

## GHRELIN A NEW HORMONE IMPLICATED IN THE REGULATION OF GROWTH HORMONE SECRETION AND BODY ENERGY HOMEOSTASIS

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### INTRODUCTION

Growth hormone (GH) has a complex regulation with two antagonistic hypothalamic hormones, growth hormone releasing hormone (GHRH) and somatostatin, as well as the liver-derived hormone IGF-I. Perhaps the old name of somatotrophic hormone (STH) is more coherent than GH, as this hormone is tightly regulated by the metabolic milieu; additionally, this regulation appears to be superimposed over the classical regulation by peptide hormones. For example, metabolic signals such as glucose, amino acids, free fatty acids and their by-products, such as keto-acids, as well as the energy balance status regulate the secretion of GH in a very relevant form. In turn, GH causes complex actions on the general metabolism of a given individual.

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### From The Editor's Desk

This issue marks the beginning of a new era for *Growth, Genetics & Hormones (GGH)*; Dr. Robert M. Blizzard retired and I became the Editor-in-Chief. This opportunity is an honor and the task is a major challenge, as it will be hard to fill the shoes of my mentor. In this issue you will find some modifications in the format and appearance of the journal and some changes in the editorial board. Hopefully, you will also note and appreciate the continuous high quality of the publication, papers reviewed and editorial comments. The lead article on Ghrelin and the abstracts along with editorial comments are very pertinent and timely, all together we are very pleased with this issue.

Dr. Judith G. Hall who had been with this journal since its inception has retired from *GGH* and will be sorely missed. I bid her farewell and thank her for all of her many contributions. On the other hand, I welcome Adda Grinberg, MD and David E. Sandberg, PhD whose abbreviated biographical sketches are posted on our web site ([www.GGHjournal.com](http://www.GGHjournal.com)). Two years ago we launched *GGH* on the internet and this has allowed us to reach a larger group of colleagues worldwide. Thus, I want to welcome a new group of international consulting editors who will help us project the journal internationally: Yoshikazu Nishi, MD in Japan, Raphael Rappaport, MD in France, and Alfonso Vargas, MD representing Latin America.

The last issue of 2003 included a survey which helped us renew our subscribers list and profile the readership. Over 60% of readers are pediatric endocrinologists and 15% are geneticists. A majority of our readers (78%) reside in the USA. Over two-thirds of the subscribers access the journal via the internet, usually after they are notified via e-mail announcing the publication of a new issue. Sixty percent view the journal and download it to keep as a reference. I thank everyone who responded to the survey and for the wonderful comments received; 81% gave *GGH* a very high/high ranking. The details of the survey are posted at [www.GGHjournal.com](http://www.GGHjournal.com).

This sets the challenge for the future - to reach more colleagues, to continue to improve the journal and to bring to our readers the most current reviews, updated information and advances in the field along with erudite editorial comments. Only through the internet with the on-line version of *GGH* can this be accomplished; within the budgetary constraints, a printed publication would not allow us to meet these goals. Thus we will continue to limit the printed subscriptions and phase out the printed journal by the end of 2004 while we further expand the readership through the internet.

Finally, I want to express my deep appreciation to our sponsors for the generous unrestricted, no strings attached educational grant for the publication of *GGH*. Please be sure you tell them how much you appreciate *GGH*. We also request that you keep us up-to-date with your current e-mail address so you continue to receive *GGH*. I thank you in advance for your consideration and comments ([editor@GGHjournal.com](mailto:editor@GGHjournal.com)).

Fima Lifshitz, MD, Editor-in-Chief

The upshot of this picture is of one hormone whose actions are implicated in a dual action on somatic growth and in the regulation of general metabolism, and which is in turn, regulated by the energetic homeostasis of the individual.<sup>1</sup> The recently discovered hormone, ghrelin, may well be the bridge connecting somatic growth with general metabolism.

## HISTORICAL BACKGROUND

Ghrelin is the result of the so called “reverse pharmacology”, which started with the development of artificial compounds named growth hormone secretagogues (GHS), followed by the cloning of their receptor and finally the identification of the natural hormone. In fact, in the late 1970s the first highly potent GH-releasing hexapeptide (GHRP-6), was developed. This was followed by other GHS compounds such as hexarelin, or MK-0677.<sup>2</sup> These GHSs were found to be potent releasers of GH *in vitro* and *in vivo*, by acting on specific receptors at the pituitary level not related to GHRH or somatostatin. Furthermore, they were active by any route of administration, including oral, and active in all the species tested. Later GHSs were used for the cloning of the GHS-receptor.<sup>3</sup> The GHSs were not discovered, but invented, as no similar compounds existed in nature. Obviously, the new receptor must have a natural endogenous ligand. The orphan-receptor strategy was then employed by the group of Kojima and Kangawa<sup>4</sup> to screen different tissue extracts. The highest expression of GHS-receptor activating factor was found in the stomach. This endogenous ligand was named ghrelin. Ghrelin was found to be a potent releaser of GH and in addition, actively participate in controlling energy balance and the regulation of food intake.<sup>5</sup> Reverse pharmacology permitted identification of this natural ligand, ghrelin.

## DISTRIBUTION OF GHRELIN-SECRETING CELLS

Two cellular areas in the body were found to be relevant in the production of ghrelin. One was an area in the gastric fundus where ghrelin is predominately expressed and secreted. Specifically, plasma ghrelin originates in the oxytic gland where A-like cells exist.<sup>6</sup> Lower concentrations have also been reported in the remainder of the bowel including the colon. Ghrelin positive cells are positioned close to the capillaries and have no contact with the lumen of the oxytic gland, indicating that secretion occurs into the plasma and not into the intestinal tract.

The second area was found in the central nervous system where neuronal cell groups release ghrelin in a synaptic transmission. Since ghrelin was determined to be implicated in the regulation of appetite, it was not surprising to find abundant ghrelin in the arcuate

nucleus of the hypothalamus which also is a region rich in GHRH neurons.<sup>4</sup> Elsewhere, in the CNS, ghrelin was also present. Immunoreactive neurons were observed in a continuum filling the internuclear space between the paraventricular, arcuate, ventromedial, and dorsomedial hypothalamic nuclei, the perifornical region, and the ependymal layer of the third ventricle.<sup>7</sup> Interestingly, these novel cell groups of ghrelin immunoreactive neurons did not overlap with any of the known cell populations implicated in energy homeostasis, thus suggesting new functions. In addition to their role in the regulation of energy balance, whether these neuronal groups also participate in the regulation of GHRH or somatostatin neurons is an open question.

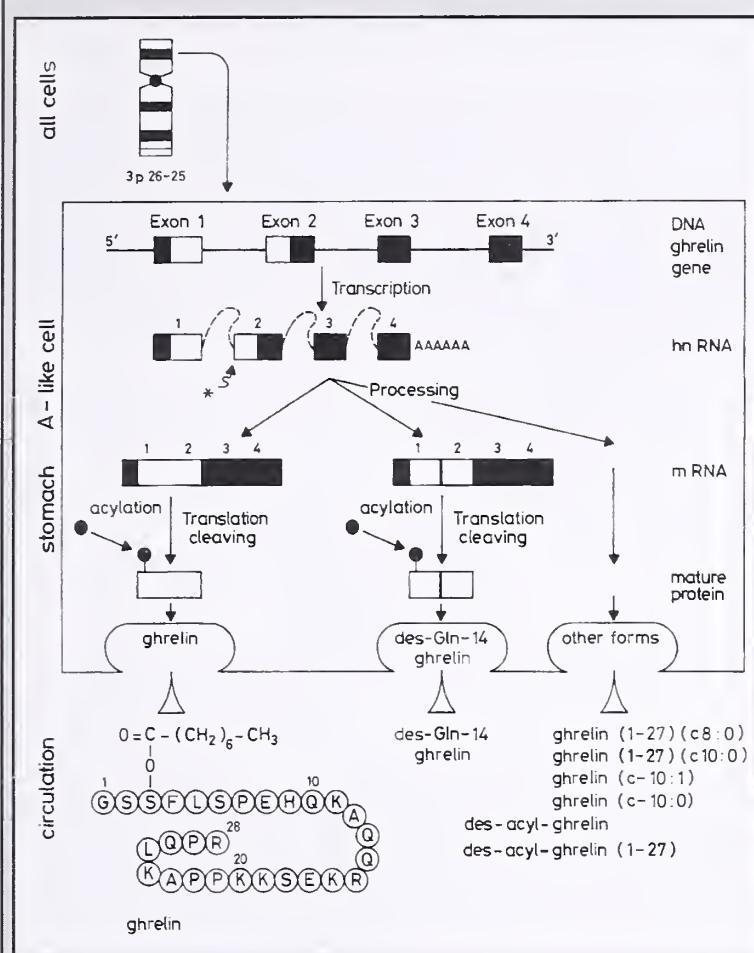
Ghrelin has also been identified in the placenta,<sup>8</sup> an organ that contains all the main regulatory components of the somatotrope axis, i.e., GH, GHRH, SST, IGF-I, and ghrelin. Although, placental expression of ghrelin changes significantly throughout pregnancy,<sup>8</sup> and is involved in the decidualization of human endometrial stromal cells,<sup>9</sup> the physiological function of this new hormone in the placenta is unknown. The pituitary, heart, kidney, endocrine pancreas, gonads, lungs, and lymphocytes all express ghrelin in low amounts.<sup>10-15</sup>

## MOLECULAR BIOLOGY

The human ghrelin gene is located in chromosome 3. It is made up of 4 exons and 3 introns. The mature protein is encoded in exons 1 and 2 (Figure 1).<sup>16</sup> The genetic structures of the ghrelin genes in the human and rat are identical and very similar to that gene in the mouse. The 5'-flanking region of the gene contains a non functional TATATAA box, as well as a ghrelin promoter which is activated by glucagon and c-AMP, although no AP1 site or CRE element is present.<sup>17</sup> Some gastric tumor cell lines express the promoter, however others do not, suggesting that human ghrelin promoter may have cell-specific activity. The hnRNA of the gene transcript is processed by alternative splicing to yield two different mature mRNAs; one produces the ghrelin precursor and the second yields des-Gln 14-ghrelin.<sup>18</sup> Ghrelin provides the first example of the production of two different mature biologically active peptides resulting from the alternative splicing of a peptide coding region.

The human ghrelin precursor (prepro-ghrelin) is composed of 117 amino acids, and the ghrelin sequence of 28 amino acids immediately follows the 23-residue signal peptide. Before being secreted, the ghrelin molecule undergoes an enzymatic process at the cytoplasm, an n-octanoyl addition at Ser 3. This esterification by n-octanoic acid, which is essential for the biological activity of ghrelin, yields the finally secreted peptide of 3315 mw. This process of acylation

Figure 1  
Operation of ghrelin gene in humans



Ghrelin is encoded in the two first exons, and it is unique in the hnRNA processing that by alternative splicing two mature mRNAs are derived, one for ghrelin and other for des-Gln-14-ghrelin. The asterisk marks the boundary between the first intron and the second exon where the alternative splicing occurs. Before secretion, a n-octanoyl acylation occurs in Ser3 through a novel and still undefined enzymatic mechanism. In addition other related molecules are also secreted in minor quantities. In rodents, a testis specific ghrelin gene-derived transcript is encoded in the third intron.

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has no precedent in cell biology either, being the first example of acylation in a secreted protein.<sup>10</sup>

The main product of that original synthesis process is mature ghrelin. The production of des-Gln14-ghrelin is minor. In addition, the human stomach releases small quantities of other related molecules.<sup>19</sup> The active binding core of the molecule consists of the first 4-5 amino acids including the acylated Ser3, short peptides containing this sequence efficiently bind to the GHS receptor, although they are devoid of GH secretory capability.<sup>10</sup> It is interesting to speculate how the fatty acid residue changes the physical properties of ghrelin to facilitate its coupling in the biomembrane-receptor structure.

## GHRELIN SECRETION AND ACTION

Ghrelin originates mainly in the stomach, and circulates at plasma concentrations of 200-600 ng/L. However, close to 80% of the total content is deamidated ghrelin, i.e., devoid of biological activity. Current RIAs mostly measure total ghrelin. Precaution is needed in the interpretation of data as bioactive ghrelin does not have a fixed ratio in relation to total ghrelin. Interestingly, the integrated secretion of ghrelin during 24 hours correlates significantly with the values obtained in the basal state<sup>20</sup> making it possible to use a single determination in clinical situations. No significant differences occur between serum and plasma concentrations; total ghrelin is resistant to repeated thawing, however warm temperatures for prolonged times should be avoided.<sup>21</sup>

Controversy exists whether ghrelin crosses the blood-brain barrier (BBB) to act as the afferent loop controlling either energy homeostasis or GH secretion. Human ghrelin has been reported to cross the BBB, but rodent

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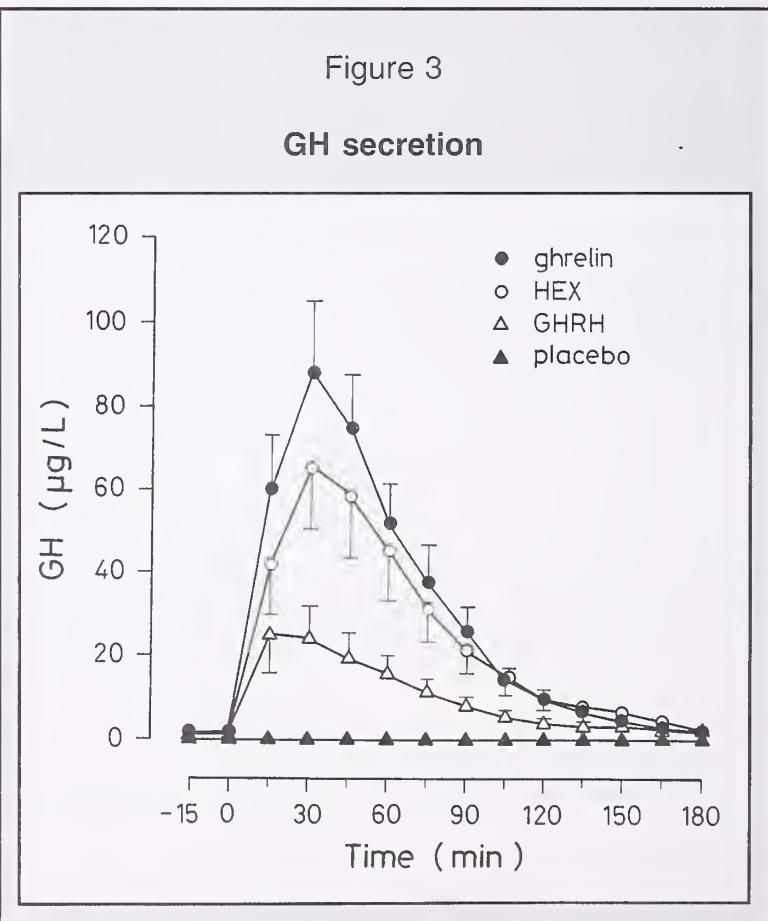
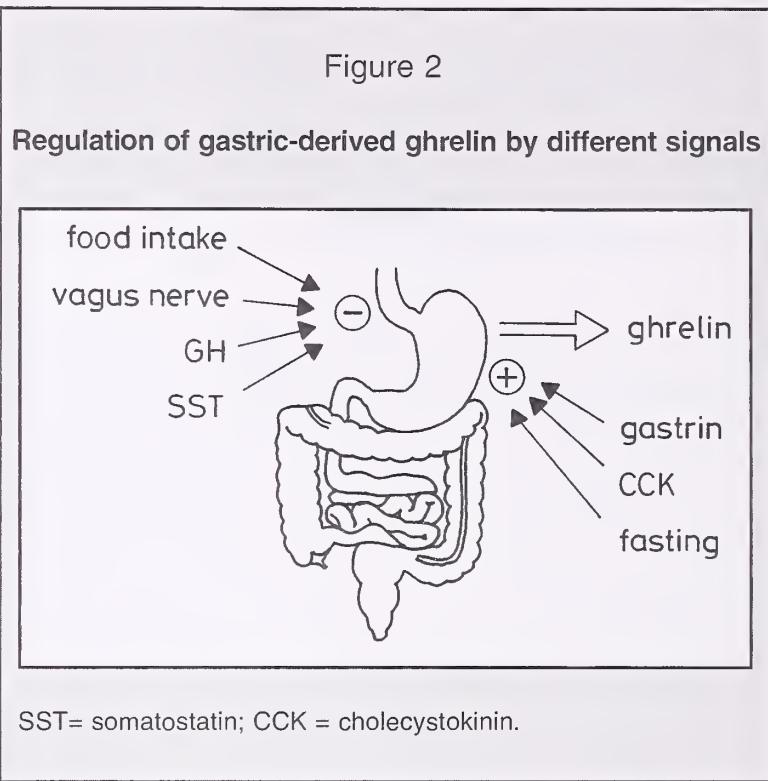
ghrelin reportedly does it with less effectiveness.<sup>22</sup> No doubt exists that ghrelin administration activates *fos* and *Egr-1* proteins in neurons of the arcuate, paraventricular and dorsomedial nuclei, and the area postrema of the hypothalamus, while deamidated ghrelin in these studies was devoid of action.<sup>23</sup> The debate is whether peripheral ghrelin acts by directly activating CNS receptors located inside or outside the BBB, or if these actions are mediated peripherally through activation of vagal nervous structures.<sup>24</sup> The latter point is of extraordinary interest as several reports state that in rats, vagotomy abolishes ghrelin-induced feeding and GH discharge. This suggests that the gastric vagal nerve is the major afferent pathway conveying ghrelin's signals to the brain.<sup>24</sup> Regardless, direct neuronal activation occurs after the activation of the ghrelin receptors, which are located on GHRH and NPY neurons, as well as in additional neurons, as was previously demonstrated for GHRP-6.<sup>23</sup> Somatostatin, cortistatin, thyroid hormones and insulin powerfully reduce gastric ghrelin secretion,<sup>25,26</sup> while cholecystokinin (CCK) and gastrin stimulate it (Figure 2).<sup>27</sup> There is no information on the regulation of ghrelin discharge by hypothalamic neurons.

Ghrelin activates the GH secretagogue receptor called GHSR-1a, a G protein coupled receptor. It activates the phospholipase C signaling route leading to an intracellular  $\text{Ca}^{2+}$  rise.<sup>9</sup> An active cross-talk at the somatotrope cell is maintained between the GHRH and the ghrelin receptors in order to coordinate and potentiate the ulterior cell response.<sup>28</sup> There is an ongoing controversy about whether the cloned secretagogue receptor is truly the receptor or just one of the receptors for that family of compounds. GHSs have specific receptors in a wide range of endocrine and non-endocrine human tissues. Most probably, different receptor subtypes exist for GHSs, with different tissue distributions.<sup>29</sup>

### GHRELIN ROLE IN THE REGULATION OF SOMATOTROPE CELL FUNCTION AND GH SECRETION

Ghrelin is a potent GH releaser in humans (Figure 3). No side-effects have been reported after the administration of large doses of this compound.<sup>30</sup> The potency of ghrelin as measured by its GH releasing capability is higher than for GHRH and comparable to synthesized GHS.<sup>9</sup> Thus, for ghrelin to be operative, the normal functioning of the GHRH receptor is necessary, as GHRH antagonists prevent or diminish the GH releasing possibilities of ghrelin.<sup>31</sup> Ghrelin is able to release GH *in vivo* when administered intravenously (IV), as well as when infused directly via the intracerebroventricular (ICV) route;<sup>27</sup> since it is able to enter the CNS from the periphery,<sup>22</sup> it is possible that

stomach-derived ghrelin may physiologically participate in GH regulation, although this has not yet been demonstrated. An important point is that ghrelin's mechanism of action is route dependent, as the vagus nerve and the arcuate nucleus are in the loop when



GH secretion in normal subjects after the administration of ghrelin, the GHS hexarelin, and GHRH (all at 1  $\mu\text{g}/\text{Kg}$  intravenously).

Redrawn from: Arvat E, et al. *J Clin Endocrinol Metab* 2001;86:1169-1174. Reprinted from: Casanueva FF, Dieguez C. *Rev Endocrine Metab Dis* 2002;3:325-338.

ghrelin is administered peripherally, but not when administered ICV.<sup>24</sup> Ghrelin-mediated GH secretion is partially insensitive to the inhibitory action of somatostatin and of metabolic compounds such as glucose or free fatty acids.<sup>25</sup> Ghrelin and GHRH showed a strong potentiation of their GH secretory capability when injected together in humans.<sup>30</sup> This peculiar activity occurs due to a simultaneous ghrelin activation of pituitary and hypothalamic structures.<sup>31</sup> There is some evidence suggesting that hypothalamic ghrelin may participate in the physiological regulation of pulsatile GH secretion.<sup>32</sup> Contrasted with the *in vitro* data, ghrelin *in vivo*, administered in what were probably pharmacological doses, induced a significant secretion of prolactin and ACTH/cortisol without altering the secretion of LH, FSH or TSH.<sup>9,30</sup> It remains to be determined what happens in respect to these responses when more physiological ghrelin doses and long-term administration are tested.

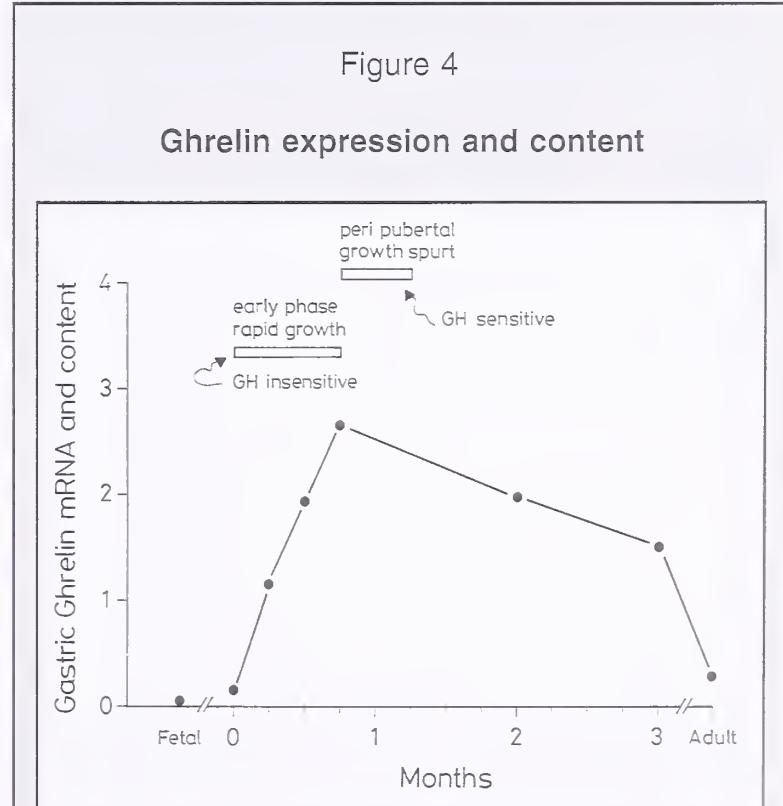
To show that IV pharmacological doses of ghrelin raise GH levels suggests, but is not proof, that ghrelin participates in the physiologic regulation of GH. A negative point is that rodents with knockout of the GHSR-1a did not show significant alterations in somatic growth, although a compensatory mechanism during fetal development may explain the lack of such results. Inferential evidence favoring a regulatory role for ghrelin, are from one side, the report of a simultaneous increase in GH and ghrelin in states of negative energy balance, and from the other the simultaneous decrease in GH and ghrelin in states of positive energy balance and obesity.<sup>9</sup> In the fetus, ghrelin mRNA is undetectable, but starts rising progressively after delivery to reach a peak at 3 weeks post-partum and it decreases thereafter.<sup>33</sup> The general pattern of ghrelin changes reminds one of similar patterns of growth rate, and GH and IGF-I secretion. Furthermore, ghrelin mRNA level increases rapidly during the early phase of rapid growth (in the 2-3 first weeks of life), a phase which is GH insensitive,<sup>34</sup> and a high level is maintained prior to and during the pubertal growth spurt which is GH sensitive (Figure 4).

In trying to understand the participation of this new hormone in the regulation of the somatotrope axis, it is worth mentioning that adult patients with GH deficiency or GH excess (i.e. acromegaly) have ghrelin levels similar to control subjects.<sup>35,36</sup> However, it may be that ghrelin plays a contributing role in the gender based differences in the pattern of GH secretion, as women in the late follicular stage have higher ghrelin levels than men.<sup>36</sup> In addition to its regulatory role on GH secretion, ghrelin has recently been reported to activate *pit-1* expression in anterior pituitary cells, an action that appears to be developmentally regulated as it is observed only in infant rats but not in adult rats.<sup>37</sup>

## GHRELIN AND THE REGULATION OF ENERGY HOMEOSTASIS

Ghrelin administration in humans powerfully induces a sensation of hunger in 75% of the subjects tested.<sup>30</sup> In rodents, ghrelin stimulates food intake while reducing fat utilization by a metabolic switch that increases the consumption of carbohydrates.<sup>38</sup> Different mechanisms than those involved in GH regulation<sup>38-40</sup> control the activity of ghrelin over food intake. Its action seems to be the exact opposite of leptin. Ghrelin is the most powerful appetite stimulant of all the known peptides; it is the unique gastrointestinal peptide that stimulates food intake. All other peptides affecting appetite are anorexigenic. Ghrelin also stimulates food intake in rodents when administered either centrally or peripherally. Other orexigenic peptides are devoid of action with peripheral administration. CNS peptides such as NPY, orexin, and agouti-related protein (AGRP) partially mediate the ghrelin action.<sup>41,42</sup>

Relevant changes in plasma levels of ghrelin appear to endorse the hypothesis that *gastric derived circulating ghrelin* regulates central appetite mechanisms. For example in rodents, ghrelin mRNA in stomach and ghrelin levels in plasma are increased by fasting and reduced by feeding, actions unrelated to gastric volume



Ontogenetic changes in ghrelin expression and content in mice gastric tissue.

Redrawn from: Liu JL, LeRoith D. *Endocrinology* 1999;140:5178-5184.

changes.<sup>38,43</sup> Passive immunoneutralization with ICV ghrelin antibodies inhibited starvation-induced as well as natural food intake in rodents, clearly indicating a tonic ghrelin action at hypothalamic receptors.<sup>44</sup> However, as blockade of the vagus nerve inhibits ghrelin-induced feeding in rodents,<sup>24</sup> perhaps peripheral ghrelin does not need to cross the BBB to activate central structures. These data do not preclude that the CNS neuronal groups secreting ghrelin may play a role, perhaps one even more relevant in the physiological regulation of appetite.

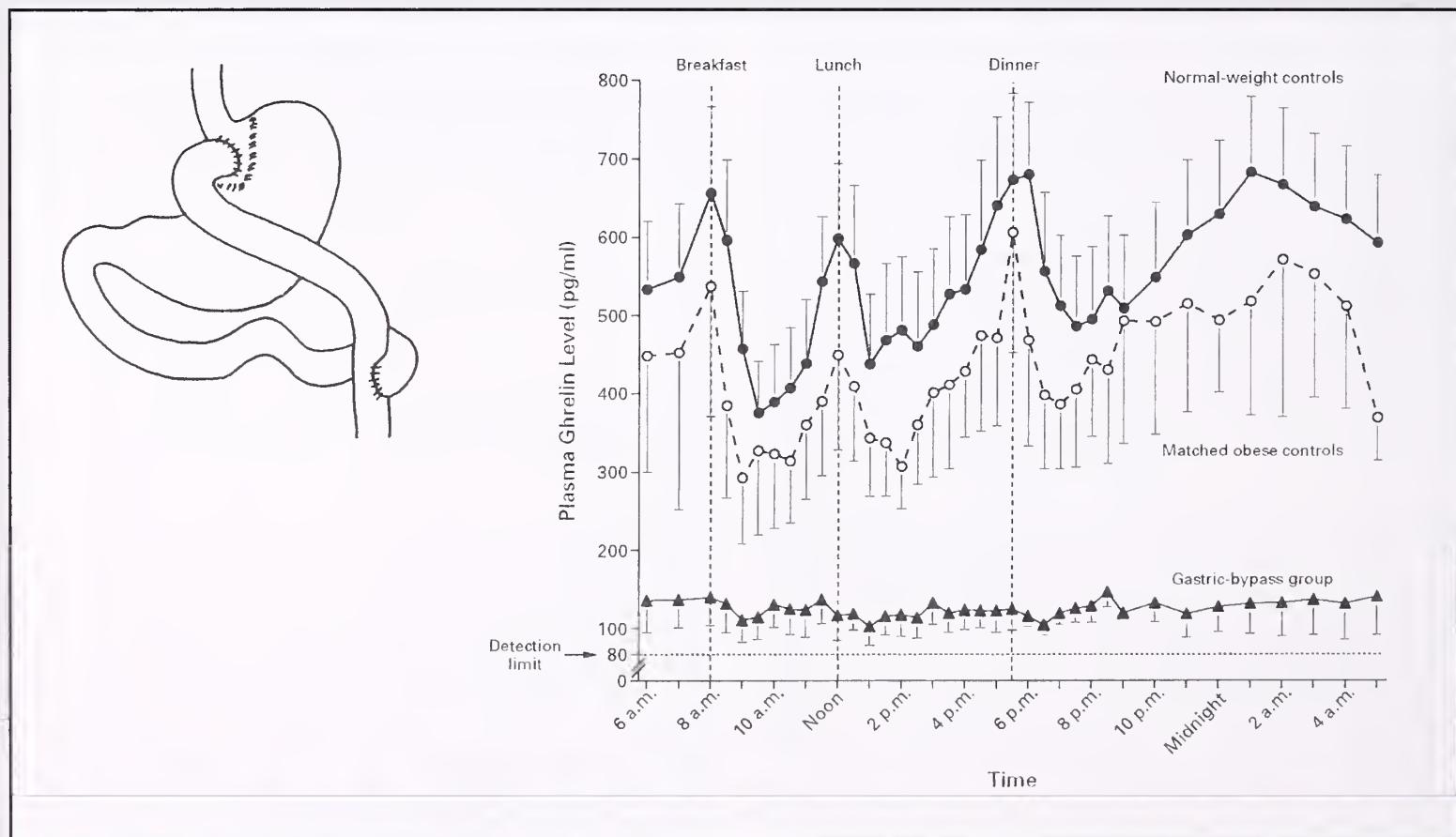
Ghrelin levels are decreased in obese subjects while elevated in states of malnutrition such as cachexia and anorexia nervosa. In the latter, weight recovery normalizes ghrelin plasma values.<sup>45</sup> In respect to the etiology of human obesity, no solid information supports its association with polymorphisms in the ghrelin gene. Circulating ghrelin undergoes relevant changes in relation to food intake, it is elevated before and decreased after feeding in a reciprocal pattern with insulin, and with intermeal changes that are in phase

with leptin.<sup>20</sup> Such results suggest that the preprandial ghrelin rise has a role in initiating meal consumption in humans. Interestingly, obese subjects who lose weight show an increase in plasma ghrelin. This fact may explain the facility of obese individuals to recover weight after dieting on the classic low-calorie diets.<sup>46</sup> Patients who have undergone bariatric surgery as treatment for obesity show a reduced ghrelin level, probably due to the absence of direct food stimulation on the gastric fundus (Figure 5).<sup>46</sup> It is a well known fact that bypass bariatric surgery is more effective over the long-term than other techniques, and that patients often refer to an absence of appetite after the surgical intervention.

Although they need to be replicated by different groups, the above results open new ways of understanding the regulation of energy homeostasis. Furthermore, the linear correlation in humans between hunger sensation and ghrelin levels, and the supranormal levels of plasma ghrelin in patients with uncontrolled hunger, such in Prader-Willy patients,<sup>47</sup> directly links ghrelin with hunger control.

Figure 5

**Circulating ghrelin levels in controls and in obese subjects**



The action of gastric bypass surgery decreases ghrelin levels.

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## GHRELIN ACTION ON OTHER HORMONAL SYSTEMS AND NON ENDOCRINE STRUCTURES

Ghrelin may also be involved in the neuroendocrine and behavioral response to stress,<sup>48</sup> and in reducing LH secretion.<sup>49</sup> Ghrelin and its functional receptor have been shown in testicular tissue to inhibit testosterone secretion, as well as in both the rat and human ovary, suggesting that ghrelin may be responsible in part for the energy homeostasis associated with control of reproduction.<sup>17,50</sup>

Ghrelin mRNA and ghrelin receptor mRNAs are expressed in gastric, thyroid, breast and lung neoplasias.<sup>15,51</sup> This opens potential new routes of treatment. Also recent data suggests that ghrelin may be an endogenous factor to promote sleep.<sup>52</sup>

In a totally different perspective, a most promising report is that both ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells.<sup>53</sup> These data support the protective actions of ghrelin on the cardiovascular system, and possibly more importantly, that there may be biological actions for the deacylated molecule.

## SUMMARY AND SPECULATION

As ghrelin anticipates the initiation of meals and releases GH, one could share the teleological view that ghrelin integrates anabolic changes in the body. In catabolic situations, raised ghrelin levels may induce a combination of enhanced food intake, increased gastric emptying and food assimilation coupled with GH levels which promote a prompt nutrient incorporation into muscles and to fat. These actions of ghrelin are the opposite of leptin which reduces food intake and selectively eliminates fat mass. Thus, both peptides may act as physiological regulators of energy balance. Interestingly, each comes from a peripheral organ (stomach and white adipose tissue, respectively). Furthermore, with conceptual incorporation of ghrelin into the group of physiological regulators of GH (i.e., GHRH, somatostatin, IGF-I), we may be on the verge of understanding better aspects of the regulation of secretion of GH that previously were not understood.

The clarification of these and other speculations are eagerly awaited. For example, it is not known if ghrelin participates in a physiological way in regulating GH secretion and energy homeostasis. If it does, it needs to be clarified whether stomach-derived circulating ghrelin and/or neuron secreted ghrelin regulate CNS food intake and GH secretion. Similarly, it is unknown whether circulating ghrelin acts after crossing the BBB, or alternatively through an unexpected mechanism related to the structure of the vagus nerve. Finally, the

part played by the scattered neuronal systems which secrete ghrelin at both hypothalamic and extrahypothalamic sites have been largely ignored for both food intake and regulation of GH secretion. Such studies will provide better knowledge of the intricate regulation of GH secretion and appetite. It can be foreseen that important new physiological insights and contributions will be provided in the future.

## ACKNOWLEDGMENTS

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## Letter to the Editor: Preterm Birth and Insulin Resistance at Adolescence

In the September issue of *GGH* (Vol 19, No 3) you reviewed an interesting publication by Singhal et al<sup>1</sup> who studied the relation between infant feeding, early growth and insulin resistance at age 13-16 years in individuals with a birth weight below 1,850 grams (which the authors labeled preterm). In their study, insulin resistance was not associated with birth weight but with growth in the first two weeks of postnatal life; thus, they concluded that Barker's hypothesis "can be reinterpreted as a postnatal event". In our opinion, their data should be interpreted more cautiously, for the following reasons.

The first point is that selection bias is quite likely. The application of birth weight instead of gestational age as inclusion criterion (< 1,850 grams) suggests that severely growth-retarded individuals born at term are also included. Another point of our concern is that in both experimental groups there were considerable numbers lost to follow-up (65-68%).

Secondly, conclusions with respect to insulin resistance in later life were drawn from a population aged 13-16 years. In this age period there is a wide variation in pubertal stages, and during pubertal development insulin sensitivity is decreased.<sup>2</sup> Moreover, girls born small for gestational age have a tendency towards early and rapid progression of puberty,<sup>3</sup> and hyperandrogenism,<sup>4</sup> which is accompanied by decreased insulin sensitivity. It is likely that many infants in the experimental groups had a low weight for gestational age at term; thus, it is conceivable that they may have shown abnormalities in pubertal onset and tempo, as well as in androgen metabolism.

Thirdly, although an earlier study of this research group (in the same population at age 7.5-8 years) suggested that suboptimal nutrition, which may result in poor early postnatal growth, adversely effects neurodevelopmental outcomes, little emphasis is put on the possible beneficial effects of nutrient-enriched preterm formulas.<sup>5</sup> This suggests that discouraging early postnatal catch-up growth by restricted food intake in infants with a birth weight below 1,850 grams is hard to justify.

The last and major point is that Singhal and colleagues have extrapolated their findings in individuals born preterm to conclusions about the general population. The first two postnatal weeks of the experimental groups took place halfway into the third trimester. In our opinion it cannot be automatically assumed that postnatal growth taking place at an age that is normally spent *in utero*, can be considered equivalent to postnatal growth of a term infant.

In conclusion, this interesting study has shown that slow early postnatal growth of preterm infants is associated with low insulin resistance at adolescence. In our view it is uncertain whether these effects persist into adulthood and whether early postnatal catch-up growth predisposes to insulin resistance only in preterm infants or also in those born at term.

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**Response:** The comments of Dr. Finken and colleagues are welcome as they point to several possible methodologic and interpretive flaws in the work of Singhal et al. Although we do not know the exact number of small for gestational age neonates included in the cohort of subjects reported, it is likely that the majority were preterm and appropriate for gestational age. If there is a potential way in which to prevent the development of insulin resistance and the dysmetabolic syndrome, it should be explored. However, clearly, one would not want to jeopardize optimal neural development under any circumstances.

Allen W. Root, MD

## ABSTRACTS

## Low-Carbohydrate Diet, Weight Loss and Cardiovascular Risk

The prevalence of childhood obesity continues to rise to epidemic proportions, with adolescents beginning to show significant signs of developing cardiovascular risk factors. A variety of weight-loss diets have been tested in adult populations, but the assessment of these diets in children, especially those with decreased carbohydrate (CHO) or fat remains limited. Sondike and colleagues report on the use of a low carbohydrate (LC) versus a low fat (LF) diet in a group of adolescents (ages 12-18) with a BMI  $>95^{\text{th}}$  percentile. Thirty-nine adolescents participated in the 12-week randomized controlled study. The LC diet consisted of a daily CHO intake of  $<20\text{g/d}$  for the initial 2 weeks and then up to  $40\text{g/d}$ . There were no restrictions on protein, fat or calories. The control group was assigned to a LF diet ( $<30\%$  energy from fat,  $<40\text{g/d}$ ) with 5 servings of starch (15g CHO each serving) daily. There were no restrictions on calories. Thirty minutes of exercise 3 times a week was encouraged, but not monitored. Subjects were weighed every 2 weeks and dietary adherence was monitored at those visits by a dietitian who reviewed 3-day food records. Lipid profiles including fasting total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol were measured along with electrolytes and liver function studies at baseline and at 12 weeks. Ketonuria was monitored and recorded by the subjects daily.

Thirty subjects completed the study (LC=16, LF=14). Subjects in the LC group lost significantly more weight than those in the LF group ( $9.9 \pm 9.3\text{kg}$  vs  $4.1 \pm 4.9\text{kg}$ ,  $p < 0.04$ ) despite having consumed more daily average calories ( $1830 \pm 615$  vs  $1100 \pm 297$ ,  $p < 0.03$ ). BMI improvement was significantly greater in the LC vs LF group as well ( $p < 0.05$ ). LF group subjects had significantly lower LDL cholesterol levels at 12 weeks than at baseline, whereas there was no change in these levels in the LC group. HDL cholesterol rose significantly in both groups and triglycerides fell significantly in the LC group. The authors state their results were consistent with those from previous weight-loss studies employing strict calorie control (protein-sparing modified fasts). Their de-emphasis on calorie control may reduce the concern for the effects of dieting on linear growth velocity. The authors also suggest that the LC diet may not be appropriate for adolescents with significant baseline elevations in LDL cholesterol. The palatability of the LC diet may be one reason that 8 of the LC subjects voluntarily remained on the diet for a year.

Sondike SB, et al. Effects of low-carbohydrate diet on weight loss and cardiovascular risk factors in overweight adolescents. *J Pediatr* 2003;142:253-258.

**First Editor's Comment:** This is an important study and hopefully it is but the first in a series of weight-loss studies designed to improve fitness and cardiovascular risk among obese children. The authors refrained from overstating their findings. As pointed out in an accompanying editorial by Daniels,<sup>1</sup> the long-term effects of LC diets on bone density, body composition, insulin resistance, and glucose metabolism remain to be defined. Sondike and colleagues do not, and because of the short 12-week duration of their study, could not address these issues. But these will need to be addressed, as will the metabolic and pathophysiologic abnormalities associated with obesity; none of these are trivial. It is anticipated that pieces of this complex "bio-psycho-behavioral" disorder will become more evident over the next few years as more and more investigators begin to study obesity and develop effective treatment regimens.

William L. Clarke, MD

**Second Editor's Comment:** The first low-carbohydrate diet for weight loss was described in 1863<sup>2</sup> and was popularized by Dr. Atkins<sup>3,4</sup> in the modern era. However, the efficacy and safety of such diets are still being debated. A systematic review of 107 articles recently concluded that there is insufficient evidence to make recommendations for or against its use.<sup>5</sup> The first randomized trial conducted for up to 12 months of such dietary therapy showed that LC diets initially induced more weight loss than the low-calorie high-carbohydrate LF diets. However at the end of one year the differences were no longer evident.<sup>6</sup> Differences in weight loss were principally associated with energy intake.<sup>7</sup> A calorie is a calorie no matter its source.

Fima Lifshitz, MD

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## Interactions in Gene Encoding Mutations Leading to Cortisone Reductase Deficiency

Draper et al studied a virilized 6-year-old boy with gonadotropin-independent isosexual precocious puberty and two adult women with polycystic ovarian syndrome (PCOS); subjects had low ratios of urinary tetrahydrocortisol to tetrahydrocortisone excretion. These findings were consistent with an autosomal recessive deficiency of cortisone reductase - the enzyme complex that interconverts cortisone (E) and cortisol (F). Cortisone reductase has dual dehydrogenase and oxoreductase activities depending on the availability of a cofactor - NADP/NADPH. There are two isozymes of 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD) - hepatic (and adipose tissue) type 1 (E $\rightarrow$ F) and renal type 2 (F $\rightarrow$ E). In previous studies, as in the present patients, the nucleotide sequence of the 6 exons of 11 $\beta$ HSD1 (chromosome 1q32-q41, OMIM 604931) was normal. However, in the three subjects in this report, mutations were found in intron 3 of 11 $\beta$ HSD1. One woman with PCOS was homozygous for double mutations - insA @ NT 83557 and T $\rightarrow$ G substitution @ NT 83597, while the second woman and the virilized boy were heterozygous for these mutations. Heterozygous carriers (parents, siblings, general population) of these linked mutations were clinically and biochemically normal. Further examination of the importance of these mutants (or polymorphic variants) revealed that the linked mutations impaired expression of 11 $\beta$ HSD1 and biologic activity of the enzyme product. Thus, the investigators concluded that intron 3 of 11 $\beta$ HSD1 served as an "intronic enhancer" of the expression of its gene.

Because the activity of 11 $\beta$ HSD1 requires a co-factor (NADPH) the authors examined NADPH generating systems and identified two mutations in the gene (*H6PD*, chromosome 1pter-p36.13, OMIM 138090) encoding the enzyme - hexose-6-phosphate dehydrogenase - that is the principle generator of NADPH in the endoplasmic reticulum in which 11 $\beta$ HSD1 is located. One mutation in *H6PD* - heterozygous 29 bp insertion between NTs 620 and 621 was present in the woman who was homozygous for the double mutation in 11 $\beta$ HSD1; a homozygous mutation - Arg453Gln - was present in the other woman with PCOS and the virilized youth. Both mutations resulted in products with substantially decreased H6PD functional activity.

## Anorectic Effects of PYY in Obesity

The gut hormone fragment peptide YY<sub>3-36</sub> (PYY) is known to reduce appetite and food intake when given to subjects of normal weight as well as to rodents. The authors investigated whether obese subjects were also sensitive to the anorectic effects of PYY. They compared the effects of this peptide by infusing it into 12 obese

The investigators concluded that inactivating mutations in both 11 $\beta$ HSD1 and *H6PD* (a total of 3 mutated alleles) must be present in order to result in sufficiently decreased 11 $\beta$ HSD1 activity to lead to the syndrome of cortisone reductase deficiency. Thus, this disorder is another example of a digenic-triallelic pattern of inheritance as are some forms of the Bardet-Biedl syndrome (OMIM 209000).<sup>1,2</sup>

Draper N, et al. Mutations in the genes encoding 11 $\beta$ -hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. *Nature Genet* 2003;34:434-439.

**Editor's Comment:** This manuscript presents yet another cause of gonadotropin-independent pseudoisosexual precocity in boys - cortisone reductase deficiency - indicating the need to measure cortisol, cortisone, and their urinary metabolites in patients with otherwise unexplained hyperandrogenic states. One wonders why females with a similar enzymatic defect do not manifest signs of hyperandrogenism until adulthood. Might there be yet another factor (gene product?) present/absent in young females that preclude early disease expression? It has been suggested that enhanced reductase activity in visceral adipose and perhaps other tissues, with consequent local hypercortisolism, might be associated with the development of visceral obesity and the "dysmetabolic syndrome".<sup>3,4</sup> Loss-of-function mutations in the gene 11 $\beta$ HSD2 (chromosome 16q12, OMIM 218030) encoding renal 11 $\beta$ HSD2 lead to hypertension in the presence of subnormal mineralocorticoid values (the syndrome of "apparent mineralocorticoid excess") because unmetabolized cortisol occupies and activates the mineralocorticoid receptor leading to renal tubular reabsorption of sodium and water and hypervolemia.

Allen W. Root, MD

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and 12 lean subjects in a double-blind, placebo-control, crossover study, and measured the effects on appetite, food intake as well as plasma levels of PYY, ghrelin, leptin and insulin. Caloric intake during a buffet lunch two hours after the infusion of PYY was significantly decreased by 30% in the obese and by 31% in the lean

subjects. PYY infusion also caused a significant decrease in the cumulative 24-hour calorie intake in both obese and lean subjects. The average decrease in the food ingestion was about one-third of the calories, as compared to the amount consumed the day prior to the infusion. However, food intake from 0-12 hours following PYY administration was more markedly reduced than that ingested from 12-24 hours after the infusion. The administration of PYY also reduced plasma levels of the appetite stimulatory hormone, ghrelin. Endogenous fasting and postprandial levels of PYY were significantly lower in obese subjects as compared to the non-obese group. Furthermore, the fasting PYY levels correlated negatively with BMI. The authors concluded that obese subjects were not resistant to the anorectic effects of PYY and suggested that a deficiency of PYY may contribute to the pathogenesis of obesity in humans.

Batterham RL, et al. Inhibition of food intake in obese subjects by peptide YY<sub>3-36</sub>. *N Engl J Med* 2003;349:941-948.

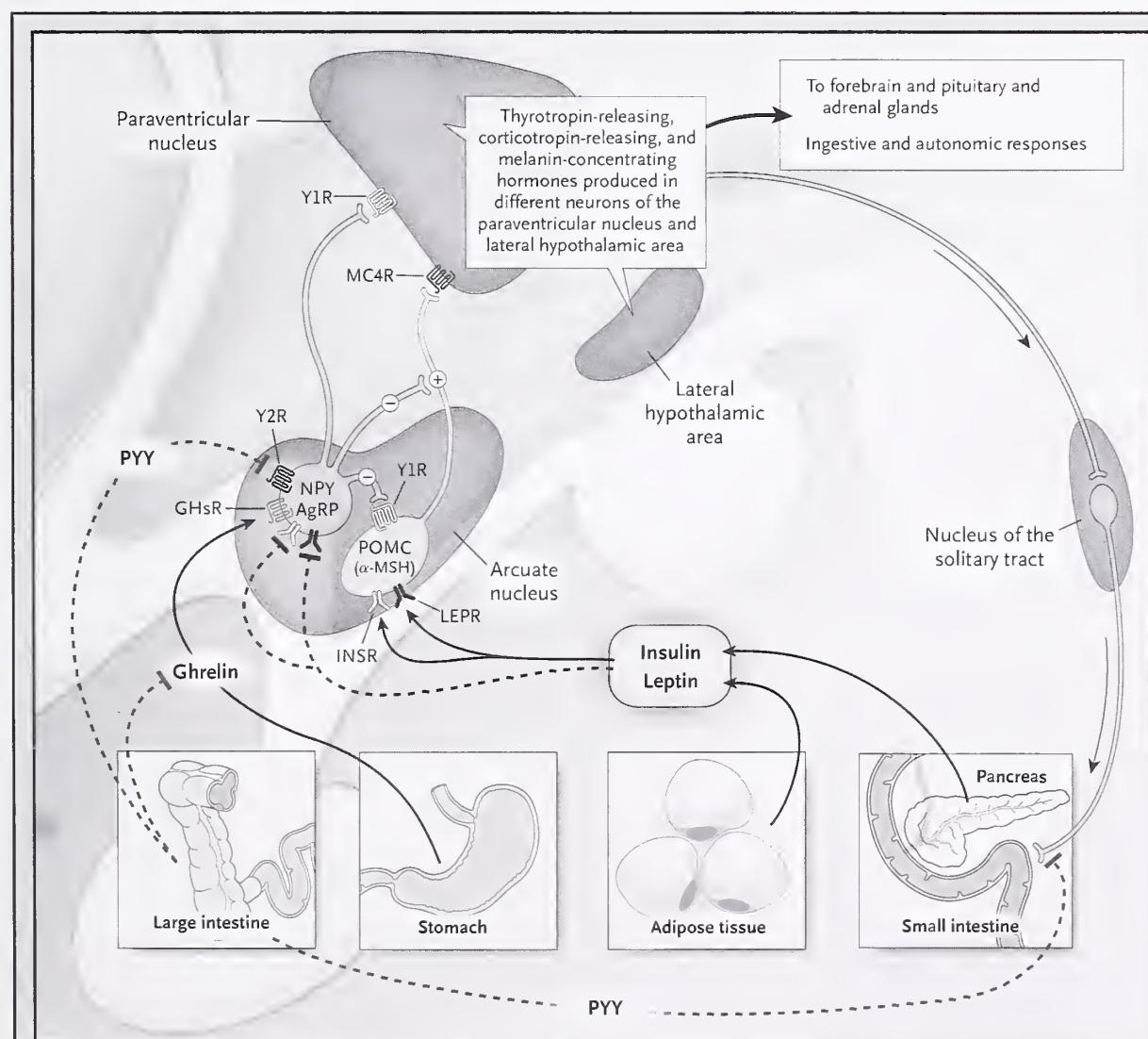
**Editor's Comment:** PYY is secreted postprandially, in proportion to the calories ingested, by endocrine L cells lining the distal small bowel and colon. PYY leads to a decrease food intake by inhibiting gut motility and increasing satiety. In this study, PYY infusion reduced hunger in both the obese and the lean individuals. These effects were directly related to the action of PYY, as there were no effects on the palatability of meals, feelings of

well being, or the presence of nausea. This peptide is one of the many signals that have been recently identified providing short-term information to the hindbrain and hypothalamus regarding hunger and satiety. Other gut hormones, such as cholecystokinin and ghrelin, also play a role in communicating with the hypothalamus and brain stem to stimulate or reduce the appetite. In this issue of GGH there is a review of ghrelin, the hunger hormone, acting on growth hormone secretagogue receptors and its pathophysiologic role in obesity related diseases.<sup>1</sup> However, the regulatory controls of food intake are more complex and involve other endocrine functions of adipose tissue, principally leptin, and appetite controlling genes, as previously reviewed.<sup>2</sup> However, PYY signal in satiety appears to play a role in obesity in humans and could be thought of as a therapeutic agent; a hope that was not realized by leptin, as in obesity there is marked resistance to the actions of this hormone. A graphic depicting the complex interactions among hormonal and neural pathways that regulate food intake and body fat mass<sup>3</sup> is shown below (Figure).

Fima Lifshitz, MD

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**Figure**  
**Interactions among Hormonal and Neural Pathways That Regulate Food Intake and Body-Fat Mass**

In this schematic diagram of the brain, the dashed lines indicate hormonal inhibitory effects, and the solid lines stimulatory effects. The paraventricular and arcuate nuclei each contain neurons that are capable of stimulating or inhibiting food intake. Y1R and Y2R denote the Y1 and Y2 subtypes of the neuropeptide Y (NPY) receptor, MC4R melanocortin 4 receptor, PYY peptide YY<sub>3-36</sub>, GHSR growth hormone secretagogue receptor, AgRP agouti-related protein, POMC proopiomelanocortin,  $\alpha$ -MSH  $\alpha$ -melanocyte-stimulating protein, LEPR leptin receptor, and INSR insulin receptor.

Reprinted with permission from Batterham RL, et al. Inhibition of food intake in obese subjects by peptide YY<sub>3-36</sub>. *N Engl J Med* 2003;349:941-948.

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## A Gene Regulator of Puberty

While evaluating a Saudi family with several first cousin marriages in which many offspring had "idiopathic hypogonadotropic hypogonadism" transmitted as an autosomal recessive trait, the authors identified a locus on chromosome 19p13.3.<sup>1,2</sup> This locus had a homozygous mutation of *GPR54* (chromosome 19p13.3, OMIM 604161, encoding an orphan G-protein receptor termed GPR54) at codon 148 in which serine was substituted for leucine (Leu148Ser). An unrelated patient was demonstrated to be a compound heterozygote with mutations in both alleles of *GPR54* - Arg331Stop leading to a truncated product and Stop399Arg - the latter resulting in an elongated protein product. *In vitro*, all mutations were found to decrease signal transduction through phospholipase C in response to the natural ligand of this receptor - kisspeptin-1 - sequence 112-121 (encoded by *KISS1*, chromosome 1q32, OMIM 603286). Kisspeptin-1 [sequence 68-121] suppresses metastases of melanoma and breast carcinoma experimentally. This 54 amino acid peptide, termed metastatin, is secreted by the placenta. In the compound heterozygotic subject, there were low basal concentrations of LH and testosterone that increased during pulsatile administration of exogenous GnRH; interestingly, this patient was more sensitive to the gonadotropin stimulating effects of GnRH than were comparable patients with hypogonadotropic hypogonadism without this specific genetic mutation.

The investigators extended these studies by developing a "knock-out" mouse model of *GPR54*<sup>-/-</sup> that reproduced the clinical picture. The *GPR54*<sup>-/-</sup> heterozygous mice had normal growth and fertility. The *GPR54*<sup>-/-</sup> deficient animals of both genders were hypogonadotropic with small gonads, hypotrophic internal genitalia, and absence of secondary sexual characteristics. Interestingly, the adrenal glands of the *GPR54*<sup>-/-</sup> animals were immature as well. Serum gonadotropin and sex hormone levels were low in *GPR54*<sup>-/-</sup> animals, but LH and FSH values increased following administration of exogenous GnRH, but the hypothalamic concentrations of GnRH were normal. The authors conclude that the kisspeptin-GPR54 system is

important in the regulation of GnRH processing or secretion in the hypothalamus rather than in the movement of GnRH secreting neurons from their embryologic site of origin in the olfactory placode (the error in Kallmann syndrome) or in the synthesis of GnRH itself.

Seminara SB, et al. The *GPR54* gene as a regulator of puberty *N Engl J Med* 2003;349:1614-1627.

**Editor's Comment:** This exciting report exemplifies the best of clinical investigation employing the most up-to-date technology in a multi-institutional collaborative that should serve as a model for future studies. The identification of a G-protein receptor (and its aptly named endogenous ligand - kisspeptin) that are involved in the regulation of GnRH release opens an entirely new control system of the reproductive endocrine axis,<sup>3</sup> a finding analogous in importance to the discovery of the role of ghrelin in the regulation of growth hormone secretion<sup>4</sup> and energy metabolism. Elucidation of the mechanism(s) by which this unit regulates GnRH secretion is eagerly anticipated. One can envision many future studies of the kisspeptin-GPR54 axis. Perhaps it is involved in the development of normal puberty. Might polymorphisms of its component genes or signal transduction system account for variations in the early or delayed onset of adolescence? Are gain-of-function mutations in *GPR54* present in some children with idiopathic central precocious puberty? Does the development of gonadotropin secreting tumors involve this pathway? Since metastatin is secreted by the placenta, this suggests that it has a physiologic role during gestation - possibly in regulation of fetal gonadotropin secretion. Future studies are eagerly and impatiently awaited.

Allen W. Root, MD

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## Growth Hormone Effects on Quality of Life of Young Adults

The investigators' goals were to document changes in quality of life (QoL) over the course of the first year post-growth hormone (GH) withdrawal, and to subsequently assess the psychological effects of reinstating GH. Participants in the GH discontinuation study were recruited from a Dutch outpatient clinic and comprised of 14 males, 8 females (ages 15 to 22 years, mean = 19 years), 11 with isolated GH deficiency (IGHD), and 11 with multiple pituitary hormone deficits (MPHD). All had

achieved adult height and were receiving adequate replacement of other hormones. Although all tested GH deficient (GHD) as children, 8 of 11 IGHD retested GH-sufficient as young adults. In contrast, all MPHD patients retested as GHD in early adulthood.

During the first six months of discontinuation of GH, a statistically significant increase in psychiatric symptoms (assessed by Hopkins Symptom Checklist) was observed, with no further increases between 6 and

12 months. There were no differences in symptoms between IGHD and MPHD, or between GHD and non-GHD. These findings corresponded temporally with a decline in IGF-I. IGF-I concentrations did not differentiate the MPHD and IGHD groups. Depressive symptoms, assessed by the Profile of Mood States (POMS), increased in both IGHD and MPHD groups by 6 months of GH discontinuation and thereafter increased further for the IGHD, but decreased within the MPHD group. The opposite pattern was observed for the POMS Tension scale, which increased across the 12 months for the MPHD group, but declined for those with IGHD. Lower IGF-I concentrations were associated with more negative mood states and somatic complaints for the combined group, whereas higher IGF-I was associated with greater 'vigor'.

Nine of 14 patients (64%; 4 males, 5 females; 2 with IGHD and 7 with MPHD) from the GH discontinuation study who remained GHD when retested as adults subsequently participated in the GH treatment study. This sample was augmented with an additional 11 patients (6 males and 5 females; 3 IGHD and 8 MPHD) who were GHD both as children and adults, had not been treated with GH in the past year, and had not participated in the GH discontinuation study. Upon reintroduction of GH to only those patients meeting adult criteria for GHD, IGF-I levels increased between 0 and 6 months in both IGHD and MPHD, but without further change by 12 months. Accompanying this increase, scores on the insecure and depression scales (of the SCL-90) decreased across the entire 12 months for both IGHD and MPHD groups, whereas anxiety (assessed by the State-Trait Anxiety Scale) decreased significantly only from baseline to 6 months. QoL scores showed a significant improvement from 0 to 6 months of GH treatment. IGF-I levels were negatively correlated with negative mood states, but positively correlated with vigor, QoL, and short-term memory. The investigators concluded that GH-modulation of IGF-I concentrations is responsible both for deteriorating mood states during GH discontinuation and improved psychological status during the return to treatment.

Stouthart PJ, et al. Quality of Life of Growth Hormone (GH) Deficient Young Adults During Discontinuation and Restart of GH Therapy. *Psychoneuroendocrinology* 2003;28:612-626.

**Editor's Comment:** As recognition has grown that the actions of GH extend beyond linear growth, the practice of treating GHD in adulthood has become more widely accepted. Unlike most studies assessing the benefits of adult GH replacement, these outcome variables were psychological rather than metabolic. In this study, both the IGHD (73% of whom retested GH-sufficient by adult criteria) and MPHD subgroups exhibited similar deterioration in emotional state upon discontinuation of GH with improvement after reinstating GH therapy. The investigators related these psychological changes to lower and subsequently improved IGF-I concentrations.

Several methodological features of this study should be taken into account before factoring them into clinical management algorithms. For instance, the investigators provide no indication of how representative study participants were of those in this clinic in meeting diagnostic and age criteria. Were those who agreed to participate more emotionally symptomatic? Research suggests considerable variability among patients in responsiveness to the QoL benefits of adult GH replacement.<sup>1,2</sup> The potential contribution of a placebo effect to mental health indices also needs to be considered. A meta-analysis suggests that placebo effects are stronger in small trials with continuous subjective outcomes.<sup>3</sup> The investigators may be attributing some psychological benefits to GH that are potentially due to response bias or placebo effect. Nonetheless this study is of great interest and provides important information.

David E. Sandberg, PhD

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## Non-Hormonal Genetic Influence on Brain Development

Current dogma holds that differences in brain development and behavior between males and females depend primarily on gonadal steroid hormones, especially testosterone and its metabolites that induce the masculine pattern and inhibit the female pattern of brain development. However, there is also evidence that genetic factors may act directly on the developing brain contributing to these differences. Until recently, this alternative view has been difficult to document, but Dewing et al provide new and convincing evidence for non-hormonal genetic effects.

Their work was done in a mouse embryo 10.5 days

after conception. This is just before the first sign of sexual differentiation of the genital ridges occurs, thus the influence of gonadal hormones could be excluded. Their strategy was to harvest whole heads from the embryos, isolate RNA into separate pools for males and females and then analyze for differential gene expression in the male and female brains. For screening analysis, they used gene (microarray) chip (Affymetrix) technology which allowed the relative expression of nearly 10,000 characterized mouse genes and over 3,000 less well defined expressed sequences (Expressed Sequence Tags – ESTs) to be determined. The normalized gene

chip results reported as fold change or difference between male and female brain RNA revealed 36 genes or ESTs with enhanced expression in females and 18 genes or ESTs with enhanced expression in males. These genes exhibited a significant fold difference of greater than 1.1 and 7 genes or ESTs for each sex displayed a fold difference of 2.0 or more. The gene showing highest differential expression in females was *Xist*, which was 18.5 fold higher in females, while genes showing the highest differential expression in males included DEAD box peptide (*Dby*) and eukaryotic translation initiation factor 2,Y (*Eif2s3Y*) with fold differences of 10.0 and 8.8, respectively. *Xist* maps to the X chromosome, while the latter two genes reside on the Y chromosome.

Real-time quantitative analysis (RT-PCR) of littermate-matched male and female embryonic brain RNA confirmed and validated the results of the gene chip screening for a small number of genes based on their potential roles in brain development. The authors concluded that developmental differences in male and female brains in mice are due in part to the differential expression of genes before gonadal secretion starts.

Dewing P, et al. Sexually dimorphic gene expression in mouse brain precedes gonadal differentiation. *Mol Brain Res* 2003;118:82-90.

**First Editor's Comment:** This is an important paper that documents the differential expression of genes in the male and female brain prior to any influence from gonadal hormones. If confirmed, it will have a substantial impact on understanding how genetic factors influence brain development. The design of the study allows for the identification of non-hormonal factors that act before the gonads are formed. However, there is no reason to think that genes act through mechanisms that do not involve gonadal hormones after gonadal hormone secretion begins, although other investigational approaches will be needed to demonstrate this. Dewing and colleagues provide no insight into the nature of the non-hormonal mechanisms through which genes may act before the appearance of gonadal hormones, although they could presumably be multiple and diverse.

One should note that the most dramatic differences were found for genes whose expression is expected to be limited to one sex or the other. For example, one would expect genes located on the Y chromosome to be expressed only in the male brain and *Xist* mRNA, which is expressed only by the inactive X chromosome in XX females, to be detected only in the female brain. That they were detected at all, seemingly reflects how the assays distinguish negative results from background signals. When these results are excluded the differences were diminished. Microarray gene chip and related approaches for studying gene expression are relatively new and evolving rapidly as is bioinformatics, the discipline that deals with analysis of the vast amounts of data this technology generates. Its novelty combined

with the complexity of its data has led to a certain amount of caution in the biomedical field with regard to the biological significance of microarray results. Initially, a 2-fold difference in expression was considered an informal threshold for biological significance. Many of the results in this study fall below this level and therefore would not be considered significant by this criteria even though they are statistically significant. However, as the analytical methods advance, the threshold is being progressively lowered such that a cut-off, such as the 1.1-fold difference used in this paper, is becoming acceptable. It is still probably wise, however, to view small differences in gene expression with caution until they are confirmed by others and placed in a biological context.

William A. Horton, MD

**Second Editor's Comment:** The findings of this study are important and exciting, and will likely contribute to a transformation of the dominant conceptual model regarding sexual differentiation of somatic phenotype, brain, and behavior. There is a risk that the findings may be misinterpreted in a manner potentially harmful to the clinical decision-making process in cases involving intersexuality. The findings force us to rethink the classic view of brain sexual differentiation and behavior which posits that the role of genes in the development of sex differences is restricted to the process of sex determination, i.e., the development of a bipotential and undifferentiated gonad into either an ovary or a testis. Evidence of a direct role of genes (not mediated by sex hormones) may lead clinicians to question the flexibility in decision-making they may currently exercise when sex assignment is in question. But should they?

The basic finding of the study is that over 50 candidate genes are differentially expressed in the brains of male and female mice, ostensibly prior to gonadal production of sex hormones. Although a remarkable observation, these findings are not necessarily relevant for one psychological outcome variable of great importance in intersex cases, that is the stability of gender identity across the lifespan. (Gender identity refers to the individual's self identification as either girl/woman or boy/man.) Readers of media reports of this article will likely draw different conclusions. The headline of one well-publicized report of this study states "Sexual Identity Hard-Wired by Genetics."<sup>1</sup> Quotes within the article imply that gender identity springs directly from our genome. If so, then how do we account for the consistent finding in the literature that 46,XY individuals with complete androgen insensitivity syndrome develop an unambiguous gender identity as girls, and later women?<sup>2</sup>

The conflict between research findings and their interpretation is likely more apparent than real and is promoted by an oversimplification of the process of psychosexual differentiation in humans. An individual's

gender identity need not be congruent with their gender-role (which refers to behaviors that differ in frequency or level between males and females in this culture and time such as toy play or maternal interest), and sexual orientation (the pattern of sexual arousal). At the present time, the clinical research literature suggests that gender identity generally conforms with the gender of rearing, even when gender assignment is discordant with genetic sex. The picture is quite different, however, with respect to the variables of gender-role behavior and sexual orientation. It is clear that many new findings will stem

from the line of research described in this report. However, it would be unfortunate if these data were to be interpreted as suggesting that gender assignment must conform with genotype to foster a stable gender identity.

David E. Sandberg, PhD

## References

1. Reuters Health. Sexual identity hard-wired by genetics. MEDLINE Plus News.
2. Hines M, et al. *Arch Sex Behav* 2003;32:93-101.

## IGF, Learning & Memory

Lupien et al tested the following hypotheses: (1) IGF treatment can prevent brain disturbances that contribute to impaired spatial learning/memory; (2) IGF-I can support cognitive function across the blood-brain barrier; (3) IGF can preserve brain function in diabetes independently of hyperglycemia; and (4) brain IGF contributes to hippocampal-based cognitive functions.

The first three hypotheses were tested by comparing normal rats versus streptozocin (STZ) diabetic rats. Four weeks after STZ, minipumps were implanted to deliver continuous infusions of 20 µg/day IGF-I or vehicle (10 mM acetic acid, pH 6.0) for 7.5 weeks. (For reference, daily IGF-I production by the adult rat liver is about 31 µg/day.) The hidden platform or "place" test was performed to assess spatial learning and memory; the "probe" test to examine memory; and the "cued" test to detect sensorimotor deficits. Following these tests, the mean blood glucose levels were  $125.0 \pm 11$  mg/dl in the non-diabetic rats versus  $515 \pm 73$  in the STZ + vehicle and  $495 \pm 99$  in the STZ + IGF rats. Body weights of both STZ groups were about half that of the non-diabetic rats.

All 3 groups decreased their latency times to escape the hidden platform, but there was a 3-day lag before latencies began to decline in the STZ + vehicle group. STZ+IGF performed similarly to the non-diabetic rats, and both groups decreased their latencies by shortening their search paths. The STZ + vehicle group decreased their latencies by increasing their swim velocity; their paths did not shorten. The average latency was more prolonged in the STZ + vehicle, than in the STZ + IGF rats. The STZ + vehicle rats also swam the furthest distance; STZ + IGF were again like the non-diabetics. Swim velocities were not significantly different, thus motor or proprioceptive disturbances were not the cause of the poorer performance of the STZ + vehicle rats. IGF infusion improved learning/memory performance without ameliorating the hyperglycemia or the catabolism of the STZ rats. Total brain weight and hippocampal weight were significantly reduced in the STZ rats, and these were not attenuated by IGF infusion. The second experimental design tested IGF's contribution to normal learning/memory by passive avoidance of electric shocks after two-weeks of continuous infusion into the lateral ventricle of either 40% anti-IGF-II

antiserum or 40% preimmune serum. Whereas the latencies of the preimmune serum rats increased, those of the IGF-II antiserum rats were significantly diminished. The authors concluded that IGF treatment can prevent brain disturbances that contribute to impaired spatial learning/memory in experimental diabetes in rats.

Lupien SB, et al. Systematic insulin-like growth factor-I administration prevents cognitive impairment in diabetic rats, and brain IGF regulates learning/memory in normal adult rats. *J Neurosci Res* 2003;74:512-523.

**Editor's Comment:** The authors integrated their results into a review of prior studies of the effects of diabetes and IGF on neurologic function. Experimentation in rats allowed controlled manipulations that cannot be made in humans, like the examination of brain tissues and the continuous intraventricular infusion of IGF antiserum. These data add to the evidence supporting IGF benefits for neurologic function. Aleman and colleagues demonstrate significant associations between circulating IGF-I concentrations and performance on perceptual-motor performance and mental processing speed in healthy men aged 65-76 years.<sup>1</sup> Although it is tempting to attribute the better performance to the higher IGF-I levels, associations are NEVER sufficient to prove causation and require corroborative evidence.

While the associations between high circulating IGF-I concentrations and increased cancer risk have garnered a lot of attention, the neurologic effects of IGF should be considered, particularly pertaining to diabetes-induced learning/memory impairments and increased risk of dementia. Gasparini and Xu recently reviewed IGF-I and insulin as it related to the pathophysiology of Alzheimer's disease.<sup>2</sup> It appears that there may also be risks to having low IGF-I levels; IGF-I does more than promote somatic growth.

Adda Grimberg, MD

## References

1. Aleman A, et al. *J Clin Endocrinol Metab* 1999;84:471-475.
2. Gasparini L, Xu H. *Trends Neurosci* 2003;26:404-406.

## Beta Cell Capacity and Insulin Sensitivity in Prepubertal Children Born Small for Gestational Age

The association between intrauterine growth retardation (IUGR) and the development of type 2 diabetes mellitus (T2DM) in adulthood has been demonstrated in several studies. Veening et al studied beta cell capacity and insulin sensitivity in 28 children born small for gestational age (SGA) and 22 children born appropriate for gestational age (AGA). All were Caucasian, born at term, and pre-pubertal (mean age 9.1 and 9.0 years, respectively). Insulin sensitivity was determined using a hyperinsulinemic-euglycemic clamp, while beta cell capacity was determined using a hyperglycemic clamp combined with arginine infusion. Anthropometric studies were obtained and relationships between catch-up growth, change in BMI, and clamp findings were determined.

Family history of T2DM and hypertension was not different between the two groups and at the time of the studies, mean actual length and BMI were similar in both groups. Insulin sensitivity was significantly lower in the SGA group. However, arginine-stimulated insulin secretion, a measure of beta cell capacity, was similar in both groups. Changes in BMI values between 0 and 1 year, 0 and 2 years, and 2 to 9 years, were categorized into tertiles. In SGA children, insulin sensitivity was significantly lower in those with the highest BMI change between years 2 to 9, compared to those with the smallest BMI change. Insulin secretion was significantly higher in SGA children with the highest BMI change in years 2 to 9, compared to those with the lowest BMI change during those years. No similar changes were seen among the responses in the AGA children.

The authors conclude that insulin sensitivity, but not beta cell capacity, is reduced in children born SGA. Thus, insulin sensitivity is the primary effect promoting the

development of T2DM in later life. But studies have shown that insulin resistance is not by itself sufficient to cause T2DM. SGA children whose BMI was greater during childhood had more insulin resistance. Thus, being overweight is clearly an important factor in the insulin resistance of SGA children and adults. The authors suggest that SGA children with excessive gain in BMI after the second year of life should be screened for the development of T2DM and associated cardiovascular risk factors.

Veening MA, et al. *Diabetes* 2003;52:1756-1760.

**Editor's Comment:** This is an important paper. These investigators have performed complex studies in a large group of SGA and AGA children and showed that insulin sensitivity rather than beta cell capacity is abnormal in the SGA children. Since we know that the risk for T2DM is increased among adults who were born SGA, and we know that T2DM requires both insulin resistance and reduced beta cell capacity, this paper implies that reduced beta cell capacity must occur later than 9 years of age. Whether reduced capacity occurs at a later age or is related in some way to increasing BMI remains to be demonstrated. The findings with regard to BMI tertiles support the need for weight control among these individuals. What role exogenous GH administration will pay in this complex metabolic process also remains to be seen. It is clearly very important that careful metabolic studies be performed in children born SGA before and during treatment with exogenous GH. Such studies should be an important part of every database that records the effects of such treatment with these children.

William L. Clarke, MD

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## CLINICAL FEATURES IN SHOX HAPLOINSUFFICIENCY: DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS

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### INTRODUCTION

The distal end of Xp and Yp is composed of 2.6 Mb DNA sequences that are identical between the X and the Y chromosome.<sup>1</sup> This particular region is named the short arm pseudoautosomal region (PAR1), where the X and the Y chromosomes recombine during male meiosis.<sup>1</sup> Since Xp terminal deletions invariably result in short stature irrespective of the breakpoints,<sup>2</sup> and small Yp terminal deletions lead to short stature,<sup>3</sup> it has been suggested that a growth gene escaping X-inactivation resides in the PAR1, and that haploinsufficiency of the growth gene causes short stature in both sexes as a dominant phenotype.<sup>2</sup>

In 1997, Rao et al<sup>4</sup> successfully cloned a novel gene at the position roughly 500 kb from the Xp/Yp telomere, and named it SHOX for short stature homeobox containing gene. SHOX consists of 7 exons and produces 2 transcripts

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### From The Editor's Desk

The miracle of the Internet has allowed the readership of *Growth, Genetics & Hormones* to grow very rapidly. We have recently added to our subscribers a substantial number of pediatric endocrinologists worldwide. Members of the Pediatric Endocrine Societies from Europe, Latin America, Colombia, and Japan who have email addresses will now be receiving *GGH* on an ongoing basis. It gives me great pleasure to welcome these pediatric endocrinologists to the family of *GGH*. Surely, our European, Latin American & Japanese contemporaries will help us broaden our perspectives and apprise us of advances in the field for publication in *GGH*. I am looking forward to contributions from our colleagues; an example of such is the lead article in this issue.

This second issue of 2004 contains a review of the clinical features of the short stature homeobox gene, so called SHOX. This important factor is implicated in the etiology of short stature and, in particular, features that characterize patients with this abnormality. This paper addresses a complicated subject, presents it in a clear easy-to-read manner, and brings the state of the art in the field to the readers of *GGH*. Drs. Tsutomu Ogata and Maki Fukami from Tokyo, Japan authored this lead article, emphasizing aspects of particular interest to pediatric endocrinologists and geneticists. The authors deserve our congratulations and thanks for their erudite writing.

This issue also contains abstracts of recent articles published in the literature that were considered of importance by our editorial board; each article is reviewed with editorial comments. Unfortunately, we have limited space and cannot publish all articles of importance in the field, nor do we attempt to do so. We limit our scope to bring value by publishing only articles that attract the interest of the editorial board and that meet our editorial standards. The high value that *GGH* has received from the readership indicates we have met our objectives, and we want to surpass them. The report of the December 2003 survey is posted at [www.GGHjournal.com](http://www.GGHjournal.com) (click on survey results). We appreciate your comments so we may continue to serve your needs.

Fima Lifshitz, MD  
Editor-in-Chief

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generated by alternative splicing of its 3' exons (SHOXa and SHOXb). SHOXa and SHOXb proteins consist of 292 and 225 amino acids, respectively. SHOXa appears to have a major biological function, although it remains to be determined whether SHOXb has some

biological role.<sup>5</sup> SHOX is expressed from an inactive X chromosome as well as an active X and a normal Y chromosome, indicating that SHOX exerts the dosage effect in sex chromosome aberrations.<sup>4</sup> Furthermore, expression analysis in human embryos has shown that SHOX is exclusively expressed in the developing distal limbs and in the first and second pharyngeal arches where Turner skeletal features are observed postnatally.<sup>6</sup>

Extensive clinical and molecular studies have demonstrated that SHOX haploinsufficiency is implicated in 2% of short stature individuals and is the predominant factor in Turner skeletal features and Léri-Weill dyschondrosteosis (LWD) characterized by Madelung deformity (shortening and bowing of the radius with dorsal subluxation of the distal ulna and partial foreleg anomalies).<sup>7</sup> In this paper, we summarize current knowledge about SHOX haploinsufficiency.

**Table 1. Skeletal features in the distal limb region in 36 patients with SHOX haploinsufficiency**

Short metacarpals and/or cubitus valgus	Madelung deformity and/or mesomelia	Prepubertal boy	Pubertal to adult male	Prepubertal girl	Pubertal to adult female
No	No	1	3	2	0
Yes	No	0	0	0	1
No	Yes	0	1	2	5
Yes	Yes	1	1	6	13

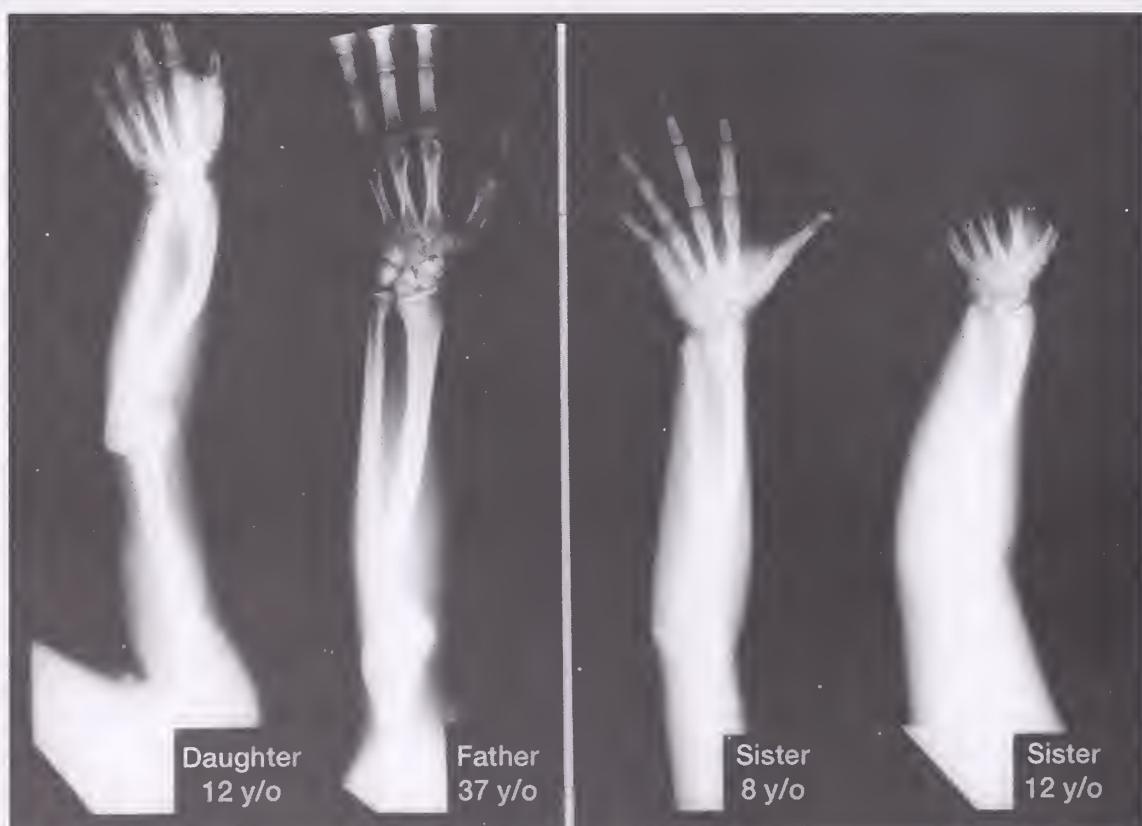
The actual number of patients is shown.

## LIMB SKELETAL FEATURES

Intragenic SHOX mutations, or pseudoautosomal microdeletions involving SHOX as the sole disease gene, have been identified in a large number of patients with normal karyotype and normal gonadal function<sup>7</sup> (also, Ogata, unpublished data). Skeletal features in the distal limb region of such individuals are classified into 4 groups on the basis of the combination of short 4th metacarpals and/or cubitus valgus which appears in 40-50% of Turner females, and Madelung deformity and/or mesomelia characteristic of LWD occurs in only approximately 7% of Turner females.<sup>8</sup> The prevalence of these features in 36 Japanese short-stature patients is shown in Table 1 (for representative roentgenograms, see Figure 1 in reference 7). These data indicated that SHOX haploinsufficiency is implicated in short stature and in the limb skeletal features of Turner and LWD patients.

Most people with SHOX haploinsufficiency have LWD features of variable extent, although there may be an ascertainment bias since patients with LWD are preferentially identified. In addition, genu valgum and relatively short lower limbs were clinically discernible in most patients with overt LWD, and tibial or fibular exostosis was occasionally detected. For the pseudoautosomal microdeletions in the telomeric part of Xp/Yp, no other features have been identified, suggesting that haploinsufficiency of pseudoautosomal genes other than SHOX has no clinical effects.<sup>9</sup>

Figure 1



Representative radiographs of the hands and forearms in 4 individuals with SHOX haploinsufficiency and normal gonadal function. The skeletal features are much more severe in the fully matured daughter with normal menses than in the father (left). The skeletal features are much more severe in the fully matured elder sister with normal menses than in the prepubertal sister with no breast development (right).

SHOX haploinsufficiency also occurs in cytogenetically discernible Xp or Yp terminal deletions. In this context,

distal limb skeletal features in 43 female patients with various types of Xp deletions involving SHOX have been summarized as follows<sup>10</sup>: (1) the prevalence of the wrist abnormality suggestive of mild Madelung deformity was significantly higher in females with spontaneous puberty than in those without spontaneous puberty; (2) the severe Madelung deformity, often detected in pubertal or adult females with normal karyotype, was not identified in these patients; and (3) the prevalence of short metacarpals and cubitus valgus was similar in females with and without spontaneous puberty (Table 2).

### Effect of Gonadal Estrogens

Limb skeletal features are more severe in females than in males, and become overt with puberty in patients with normal karyotype (Figure 1). The so-called idiopathic short-stature phenotype was predominantly exhibited by male patients and prepubertal girls, and LWD was predominantly manifested by pubertal and adult female patients (Table 1). In this context, two matters are noteworthy. First, SHOX appears to function as a repressor of growth plate fusion and skeletal maturation in the distal limb region, so that SHOX haploinsufficiency results in premature growth plate fusion and relatively advanced skeletal maturation in that region.<sup>11,12</sup> Second, skeletal maturation in normal individuals is primarily caused by gonadal estrogens—which increase with puberty—serum estrogen levels being higher in females than in males.<sup>13</sup> Thus, it is likely that gonadal estrogens exert a maturational effect on skeletal tissues that are susceptible to unbalanced premature growth plate fusion, facilitating the development of skeletal lesions in a female-dominant and in a pubertal tempo-influenced fashion.<sup>7,13</sup> Furthermore, the tempo of pubertal development may also play an important role in the development of skeletal features. Severe forms manifested in early maturing girls who are exposed to gonadal estrogens from a relatively early age.<sup>13</sup> This may also account for the sex differences in the severity of skeletal features in SHOX haploinsufficiency, because females enter puberty approximately 2 years earlier than males. Thus, it is inferred that, in SHOX haploinsufficiency, the amount and tempo of gonadal estrogen production in females usually cause LWD, whereas those in males usually lead to the so-called idiopathic short-stature phenotype.

This notion is consistent with the findings in female patients with cytogenetically recognizable Xp deletions. The wrist abnormality is predominantly manifested by females with spontaneous puberty, and the absence of severe Madelung deformity is compatible with a relatively small amount and slow tempo of gonadal estrogen production.<sup>9</sup> Furthermore, this idea is applicable to Turner syndrome as well: (1) the prevalence of Madelung deformity is only approximately 7%,<sup>8</sup> and this relatively low prevalence would be explained by the compromised gonadal estrogen of these patients (Table 3); (2) the

**Table 2. The prevalence of Turner skeletal features in 43 patients with Xp deletions involving SHOX**

	Lymphogenic gene(s)¶		Spontaneous puberty	
	Preserved	Deleted	Positive	Negative
Distal limb region‡	11/19	19/24	*17/20	*13/23
Short metacarpals	7/19	12/24	12/20	7/23
Cubitus valgus	9/19	18/24	15/20	12/23
Wrist abnormality#	*8/18	*2/23	*9/18	*1/23
Faciocervical region‡	4/19	10/24	6/20	8/23
High arched palate	3/18	8/23	4/18	7/23
Short neck	*1/19	*7/24	4/20	4/23

Denominators indicate the number of patients searched for each feature.

\* P<0.05 and †P<0.01 by the Fisher's exact probability test.

‡ The ratio of patients with at least one feature of each category.

# Presence of at least one of the following features: decreased carpal angle, metaphyseal lucency and/or epiphyseal hypoplasia at the ulnar side of the distal radius, and angulation of the distal radius and/or ulna.

¶ Lymphogenic gene indicates the gene involved in lymphatic development located between DMD and MAOA on Xp.

prevalence of spontaneous genital bleeding in Turner syndrome patients is higher than that of Madelung deformity (15%–20% vs 7.5%),<sup>8,14,15</sup> which could be ascribed to relatively small amounts and slow tempo of gonadal estrogen production<sup>14,15</sup>; and (3) estrogen treatment in Turner syndrome does not increase the prevalence of Madelung deformity because this therapy is usually started in late teens with a low dosage and for short periods.<sup>16</sup>

Of the skeletal features in the distal limb region, short metacarpals and cubitus valgus are frequently exhibited in those with Turner syndrome who have gonadal estrogen deficiency. These remain relatively infrequent in patients with SHOX haploinsufficiency who have normal gonadal function (Table 3). This may imply that such skeletal features are also caused by additional factors other than gonadal estrogens. One possibility would be a compressive effect of peripheral lymphedema resulting from haploinsufficiency of the lymphogenic gene (for lymphogenic gene, see below).<sup>17</sup> In support of this, Noonan syndrome patients often have such skeletal features in the presence of peripheral lymphatic malformation.<sup>18,19</sup>

### FACIOCERVICAL SKELETAL FEATURES

Faciocervical skeletal features are occasionally manifested in patients with SHOX haploinsufficiency and normal karyotype (Table 3). In addition, short neck has been described in German subjects.<sup>6,13</sup> This suggests that SHOX haploinsufficiency may also be relevant to the skeletal features in the faciocervical region of Turner syndrome patients. However, the prevalence of these features is apparently lower than that of limb skeletal features in patients with normal karyotype. Furthermore, the prevalence of faciocervical skeletal features is apparently lower in patients with normal

karyotype than in patients with Turner syndrome (Table 3). In particular, micrognathia occurs in approximately 60% of Turner syndrome females, but is rare in patients with normal karyotype.

In contrast, faciocervical skeletal features are frequently exhibited by females with Xp deletions involving SHOX. In this respect, the data are summarized as follows<sup>9</sup>: (1) the prevalence, especially that of short neck, is higher in females with large Xp deletions presumably missing the putative lymphogenic gene (see below) than in those with small Xp deletions presumably preserving that gene; and (2) the prevalence of high-arched palate is similar among females with and without spontaneous puberty, as is that of short neck (Table 2).

### Relevance of the Lymphogenic Gene

Turner syndrome is associated with lymphatic hypoplasia.<sup>20,21</sup> This postulates that a lymphogenic gene escaping X-inactivation may be shared by the X and the Y chromosome, and that haploinsufficiency of the gene results in lymphatic hypoplasia as a dominant phenotype. The lymphogenic gene has been mapped to an approximate 9-Mb region between DMD and MAOA on Xp and to an approximate 4-Mb region between PABY and DYS255 on Yp, by genotype-phenotype correlations.<sup>9,22</sup>

Lymphatic hypoplasia leads to lymph fluid stasis, resulting in distension of the main and tributary lymphatic ducts and in lymphedema. Thus, a mechanical force would be exerted on tissues and organs adjacent to the lymphatic system. It is hypothesized that soft tissue and visceral stigmata are deformational consequences caused by the mechanical force of distended lymphatics and lymphedema.<sup>17</sup> Indeed, it appears reasonable to assume that a distended cervical lymphatic system (cystic hygroma) leads to nuchal region anomalies such as webbed neck and low posterior hairline, and that peripheral lymphedema results in acral region anomalies such as puffy hands and feet and redundant skin.<sup>17,23</sup> It also appears reasonable to postulate that cystic hygroma and distended thoracic and para-aortic lymphatic ducts compress the aortic arch and alter the cardiac hemodynamics, leading to cardiovascular anomalies such as aortic coarctation, and that distended

retroabdominal and iliac lymphatic ducts inhibit normal upward migration and rotation of the kidney, leading to renal malformations such as horseshoe kidney.<sup>17,24,25</sup> It is notable that visceral anomalies in Turner syndrome are limited to the organs in the vicinity of the main lymphatics. Thus, characteristic soft tissue and visceral anomalies can be regarded as the result of a malformation sequence initiated by lymphatic hypoplasia.<sup>17</sup>

By analogy, it is inferred that cystic hygroma and facial edema exert a compressive effect on the developing faciocervical skeletal tissues primarily in the fetal life, facilitating the development of faciocervical skeletal features of SHOX haploinsufficiency. This notion implies that haploinsufficiency of the lymphogenic gene, rather than gonadal estrogens, is relevant to the development of faciocervical skeletal features. This hypothesis explains the difference in the prevalence of faciocervical skeletal features between patients with Turner syndrome and those with SHOX haploinsufficiency (Table 3). Furthermore, since lymphatic distension occurs in the peripheral areas including distal limb regions, this would contribute to the development of cubitus valgus and short metacarpals in Turner syndrome (Table 2).<sup>19</sup>

### GROWTH PATTERNS

#### Patients With Normal Karyotype

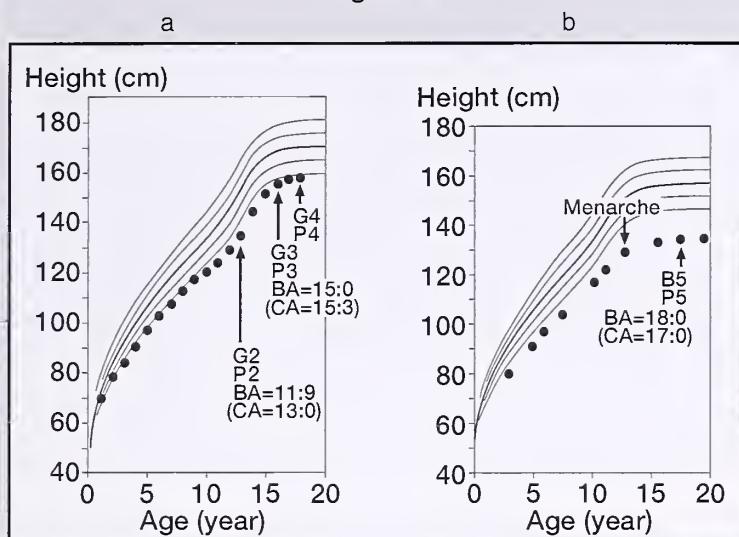
Patients without overt LWD usually grow along the -2 SD growth curve throughout the growth period (Figure 2a).<sup>13</sup> The magnitude of the height deficit is compatible with the previous estimation that loss of SHOX decreases the adult height by about 12 cm in the absence of overt LWD.<sup>26</sup> This difference in size approximates the magnitude of 2 SD of the adult height in the normal population. This implies that SHOX haploinsufficiency leads to short stature (<2 SD) in roughly half of patients without LWD (approximately 50% of penetrance). Indeed, normal stature has been described in several patients with SHOX haploinsufficiency.<sup>13</sup> In this regard, since normal height has been observed in patients born to tall parents,<sup>13</sup> this implies that statural growth in patients with SHOX haploinsufficiency is influenced by original height potential as represented by the parental height, as has been reported in Turner syndrome.<sup>27</sup> It remains to be clarified, however, how SHOX haploinsufficiency causes the idiopathic short-stature phenotype.

Table 3. The prevalence of skeletal features

	SHOX haploinsufficiency (normal karyotype)	Turner syndrome (abnormal karyotype)
Distal limb region		
Short metacarpals, cubitus valgus	18%	40%
Madelung deformity, mesomelia	75%	7%
Faciocervical region		
Short neck	5%	40%
High arched palate	8%	35%
Micrognathia	0%	60%

Adopted from ref. 7, 8, and unpublished observations.

Figure 2



Growth charts in patients with proven SHOX haploinsufficiency and normal gonadal function. The actual heights are plotted on the sex-matched standard longitudinal growth curves (the mean,  $\pm 1$  SD, and  $\pm 2$  SD) for Japanese children. Pubertal stage is indicated according to the classification of Tanner (G: genitalia; B: breast; P: pubic hair). Bone ages (BAs) are given, together with chronological ages (CAs) at the time of BA determination. A boy without recognizable skeletal abnormalities (left), and a girl with short 4th metacarpals, cubitus valgus, and dyschondrosteosis characterized by Madelung deformity (right).

explained by assuming that prepubertal growth is relatively well preserved because of dormant gonadal function, whereas pubertal growth is compromised because of production of gonadal estrogens which facilitate growth plate fusion.<sup>13</sup> The severely affected final height suggests that SHOX haploinsufficiency causes short adult height ( $<-2$  SD) in most patients with overt LWD (probably approximately 70% of penetrance). Furthermore, consistent with the SHOX expression pattern, the longitudinal growth study of patients with SHOX haploinsufficiency and normal gonadal function showed that sitting height was fairly stable throughout the growth period, whereas leg length and arm span were compromised during puberty, thereby worsening mesomelic short stature.<sup>28</sup>

### Patients With Turner Syndrome

SHOX haploinsufficiency alone is unlikely to explain the growth failure and the growth pattern of 45,X Turner syndrome.<sup>2,13</sup> In 45,X Turner syndrome, the mean adult height is about  $-3.2$  SD below the mean of normal females, and the linear growth is associated with a reduced growth rate beginning in early childhood, in the absence of discernible LWD.<sup>2,29</sup> It is noteworthy that 45,X is associated with a gross chromosome imbalance, which has been suggested to result in global developmental defects, including growth failure.<sup>30,31</sup> Although 45,X Turner syndrome females usually have gonadal dysgenesis, gonadal estrogen deficiency is unlikely to influence adult height or childhood growth patterns.<sup>2</sup> Thus, the remaining

growth deficit and the reduced growth rate from early childhood in 45,X Turner syndrome appears to be due to chromosomal imbalance.

One may argue that severe short stature in Turner syndrome is contributed by loss of another growth gene(s) escaping X-inactivation. However, such a growth gene(s) other than SHOX is unlikely to exist on the X chromosome [for details, see Reference 2], although the possibility that a growth gene(s) escaping X-inactivation might exist on Xp has not been excluded.<sup>2,32</sup> The adult height is similar between apparently non-mosaic Caucasian females with 45,X, those with 46,X,del(X)(p11), and those with 46,X,i(Xq); this argues against the presence of a growth gene escaping X-inactivation on Xq.<sup>11</sup> Thus, the shorter mean adult height in patients with larger Xq deletions than in those with small Xq deletions is inexplicable without assuming the growth disadvantage of a chromosomal imbalance.<sup>2</sup> Similarly, the shorter mean adult height in patients with larger Xp deletions than in those with small Xp deletions would also be ascribed to the growth disadvantage of a chromosomal imbalance, rather than to loss of a growth gene on Xp escaping X-inactivation.<sup>2</sup> In addition, short stature in apparently non-mosaic Caucasian females with 46,X,idic(Xp) missing SHOX suggests that a growth gene escaping X-inactivation is absent from most of Xp.<sup>2</sup>

### DIAGNOSTIC IMPLICATIONS

#### Prevalence

The prevalence of SHOX haploinsufficiency has been estimated to be approximately 2% in individuals with normal karyotype with the so-called idiopathic short-stature phenotype ( $<-2$  SD).<sup>4,33,34</sup> However, re-examination of such patients has frequently disclosed mild skeletal abnormalities such as decreased carpal angle, angulation of distal radius, tubular bone alterations, and brachymetacarpia.<sup>35,36</sup> Furthermore, the prevalence should be different between sexes and ages, since normal skeletal features are predominantly exhibited in males of various ages and in prepubertal girls.<sup>7,13</sup> Thus, further studies are necessary to estimate the sex- and age-specific prevalence of these alterations in the so-called idiopathic short-stature phenotype.

The prevalence of SHOX haploinsufficiency is 80% to 90% in patients with normal karyotype and LWD (reviewed in Reference 7), with the lowest value of 60%<sup>37</sup> and the highest value of 100%.<sup>38</sup> Although SHOX haploinsufficiency remains undetected in a small fraction of patients with LWD, it is unknown at this time whether LWD is a genetically heterozygous condition caused by a hitherto unknown autosomal gene(s), or if SHOX mutations reside in the unexamined regions, such as the promoter and enhancer sequences.

In contrast to LWD, SHOX haploinsufficiency is rarely found in normal karyotype patients with short metacarpals and/or cubitus valgus, but without LWD phenotype. To date, only 1 female has been identified with this pattern of phenotype.<sup>13</sup> This would not be surprising, however, because short metacarpals and cubitus valgus appear to be highly heterogeneous conditions and could occur as a normal variant phenotype.

### Clinical Indications

Phenotypic assessment in SHOX haploinsufficiency provides useful clues for the selection of normal karyotype patients to be studied for this condition. First, patients with LWD phenotype should be sought. In this context, of practical importance is to recognize the signs of Madelung deformity on hand and wrist radiographs that are almost invariably obtained for the bone age evaluation in children with short stature. For this purpose, it is recommended to carefully observe the signs of Madelung deformity, such as metaphyseal lucency and epiphyseal hypoplasia at the ulnar border of the distal radius, decreased carpal angle, angulation of the distal radius and ulna, and subluxation of the distal ulna.<sup>7,39,40</sup> In our experience, the first signs of Madelung deformity are often exhibited by metaphyseal lucency and epiphyseal hypoplasia of the medial side of the distal radius in prepubertal patients, as well as by decreased carpal angle in pubertal or adult patients (Figure 3). When such findings are suspected, radiographs of the distal limbs should be obtained in order to search for characteristic features such as radial curvature and shortening. Second, SHOX haploinsufficiency should also be considered for patients with mesomelic short stature, which becomes evident in puberty. Third, familial members of a proband with SHOX haploinsufficiency should be studied irrespective of clinical phenotype. Indeed, familial studies have identified SHOX haploinsufficiency in subjects—especially males—with low-normal height alone.<sup>13</sup>

### Molecular Diagnosis

Molecular studies are necessary to identify SHOX haploinsufficiency, especially in patients with normal karyotype. In this context, it is noteworthy that microdeletions involving SHOX are much more prevalent than intragenic SHOX mutations.<sup>7</sup> The high prevalence of microdeletions would be consistent with repetitive sequences such as subtelomeric interspersed repeats being abundantly present around SHOX,<sup>41</sup> because an unequal crossing over between homologous chromosomes or an intrachromosomal recombination is prone to occur in such a region.

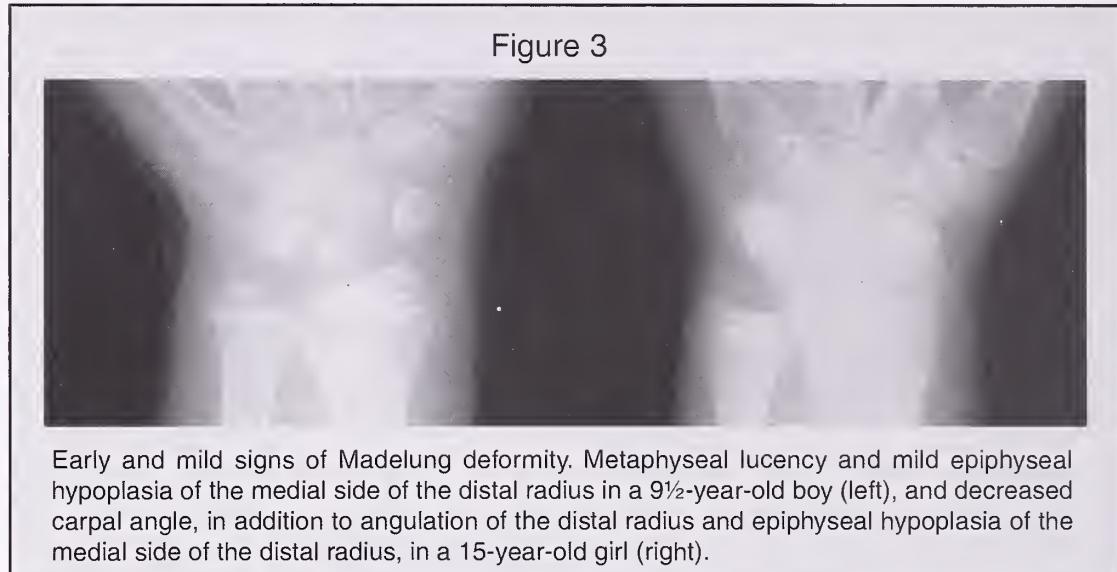


Figure 3

Early and mild signs of Madelung deformity. Metaphyseal lucency and mild epiphyseal hypoplasia of the medial side of the distal radius in a 9½-year-old boy (left), and decreased carpal angle, in addition to angulation of the distal radius and epiphyseal hypoplasia of the medial side of the distal radius, in a 15-year-old girl (right).

Thus, it is recommended to search for a SHOX deletion first, and when SHOX deletion is excluded, an intragenic mutation should be investigated. For SHOX deletion analysis, fluorescence *in situ* hybridization is recommended because it unequivocally shows the presence or absence of SHOX. Microsatellite analysis for the CA repeat marker at the 3' region of SHOX is also useful because of its high heterozygosity (>90% in our experience), although parental DNA is necessary to confirm SHOX deletion. For SHOX mutational analysis, sequence analysis is essential. In this respect, denaturing high performance liquid chromatography analysis serves as a rapid and reliable screening method.

### THERAPEUTIC IMPLICATIONS

#### Growth Hormone

Growth hormone (GH) therapy may be advantageous in SHOX haploinsufficiency because it is effective in Turner syndrome, despite the absence of GH deficiency. Indeed, beneficial short-term effects have been reported in several patients.<sup>33,42,43</sup> However, GH therapy might facilitate the development of skeletal anomalies by accelerating distorted skeletal growth resulting from unbalanced premature fusion, or by stimulating gonadal development and resultant estrogen production.<sup>42</sup> Therefore, careful follow-up is required for GH therapy in SHOX haploinsufficiency.

#### Gonadotropin-Releasing Hormone Analog

Gonadotropin-releasing hormone analog (GnRHa) therapy is expected to serve as prevention or mitigation of the development of skeletal features by suppressing gonadal estrogen production.<sup>42</sup> However, GnRHa therapy has performed poorly in SHOX haploinsufficiency, so that the adequate timing to start and stop the GnRHa therapy is unknown. At present, it may be recommended to attempt GnRHa treatment in an experimental protocol for early maturing girls or in patients with early signs of Madelung deformity, possibly in combination with GH.

## SUMMARY AND SPECULATION

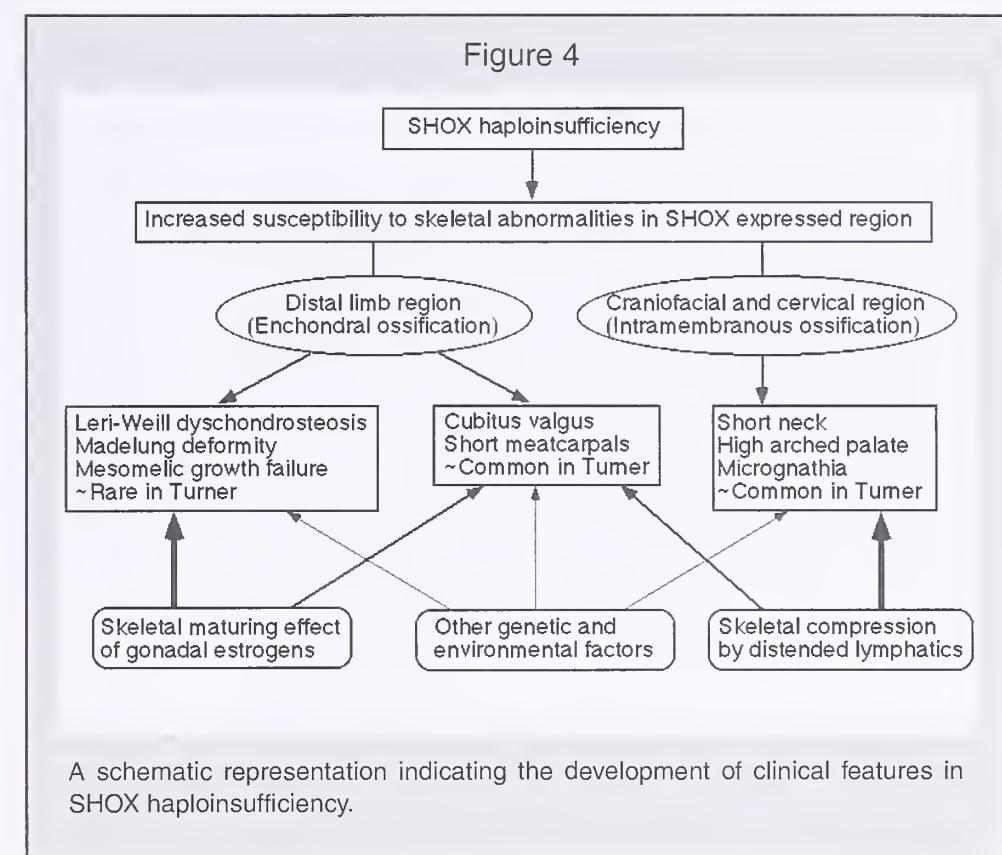
Clinical studies have indicated that SHOX haploinsufficiency is responsible for not only short stature but also Turner syndrome skeletal features and LWD. The expressivity of SHOX haploinsufficiency in the limb and faciocervical regions is primarily influenced by gonadal function status and the presence or absence of the lymphogenic gene, respectively (Figure 4). In this context, although phenotypic spectrum in diseases resulting from haploinsufficiency of transcription factor genes is known to range widely from nearly normal to severely affected phenotypes<sup>44</sup> (a list of haploinsufficiency is given in Reference 45), the underlying factor(s) for clinical diversity remains unknown in nearly all such diseases. Thus, SHOX appears to be the first gene in which modifying factors for haploinsufficient status have been identified.

Finally, two points should be made with respect to SHOX. First, a gene(s) that controls SHOX expression is unknown, as is that controlled by SHOX. Identification of such upstream and downstream genes should serve to facilitate an understanding of the molecular network of human growth. Second, it has been shown that SHOX overdose in association with gonadal dysgenesis constitutes a novel clinical entity leading to tall stature at pubertal age in normal children.<sup>7</sup> Because gonadal estrogen production can be suppressed by GnRHa therapy, this may argue for the possibility of a SHOX gene therapy in patients with growth failure. Further accumulation of clinical and molecular data will provide better clues for the diagnosis and management of SHOX haploinsufficiency.

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Figure 4



A schematic representation indicating the development of clinical features in SHOX haploinsufficiency.

## LETTER TO THE EDITOR

## Sexual Outlook for Post-Surgical Ambiguous Genitalia Patients

The December 2003 edition of *Growth, Genetics & Hormones* (Vol. 19, No. 4) abstracted, "The effects of clitoral surgery on sexual outcome in individuals who have intersex conditions with ambiguous genitalia: a cross-sectional survey."<sup>1</sup> This was a postal survey of the sexual function of 39 adults born with ambiguous genitalia, reared as girls. Those who had undergone clitoral surgery reported more sexual difficulties than those without surgery. The authors concluded, "Adult sexual function could be compromised by feminizing genital surgery." An editorial comment emphasized this finding, "The challenge is to devise a corrective procedure that does not do so." The implication is that such a procedure will be surgical in nature.

We wonder whether determination of a satisfying sexual outcome is more complex than this. Both surgical and non-surgical groups reported significantly more problems in several sexual domains than the general population sample. Sexual dysfunctions (particularly sexual aversion disorder and sexual pain disorders) are more common in women who have had genital surgery.<sup>2</sup> Surgical damage to the autonomic pelvic network may lead to iatrogenic sexual dysfunctions.<sup>3</sup> However, Bancroft et al<sup>4</sup> reported that physical aspects of sexual response in women (including arousal and orgasm) were poor statistical predictors of sexual satisfaction: "The best predictors of sexual distress were markers of general emotional well-being," though other variables such as mood, body satisfaction, sexual knowledge, and confidence may also play a role.<sup>1</sup> The authors acknowledge that the poor sexual outlook for intersexed adults may be related to psychological factors as much as to surgical sequelae.

In an accompanying commentary, Slijper<sup>5</sup> comments on some of the psychological factors that modulate sexual functioning, including "sexual shyness" (which could be caused by dissatisfaction with the appearance of the genitals) and "gender behavior" (referring to masculine behavior in XY children assigned female sex). She advocates counseling prior to the onset of puberty to reduce the impact of these factors as a treatment strategy to enhance sexual satisfaction through comfort with one's body and gender assignment. Slijper's authority derives from her membership in a "Gender Team" at a children's hospital in the Netherlands. Unfortunately, there are very few centers such as this, where patients born with disorders of sexual differentiation will receive specialized psychoendocrine treatment. It is possible that such an integrated approach (medical, surgical, and psychological) will result in more positive outcomes for these individuals.

David E. Sandberg, PhD  
Buffalo, New York

Nina Williams, PsyD  
Highland Park, New Jersey

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4. Bancroft J, Loftus J, Long JS. *Arch Sex Behav*. 2003;32:193-208.
5. Slijper FM. *Lancet*. 2003;361:1236-1237.

**First Editor's Comment:** This writer strongly believes that it is inappropriate to rear genotypic and potentially fertile girls with ambiguous or fully masculinized external genitalia as males under most circumstances. The neonate does not exist in isolation but as a member of a family in which the birth of an infant with ambiguous genitalia causes unimaginable stress that can be alleviated only to a modest extent by education, conversation, and reassurance. In most families, corrective genital surgery to conform with the selected sex of rearing is desired as soon as possible. Comparing (admittedly anecdotal and by personal experience) family outcomes in the decades when clitoral surgery in girls with ambiguous genitalia was performed between 2-5 years of age and the current practice of clitoral recession within the neonatal period, far more marital conflicts (spousal abuse, separation, divorce) arose with the delayed rather than early clitoral surgery. Recognizing that corrective genital surgery will be undertaken on the majority of girls with ambiguous genitalia, development of a surgical procedure that will both normalize genital appearance and maintain genital sensation is ideal. Certainly, long-term monitoring and counseling of the family and patient is highly desirable if the facility and personnel to do so are readily available and freely accessible. I agree that we have learned over the past several decades that the most important sex organ is the brain and that normalization of genitalia will not ensure "sexual satisfaction"; even in the normal female population there appears to be a surprising degree of sexual dissatisfaction – a driving force behind the pharmaceutical industry's effort to develop a "female sindenafil".

Allen W. Root, MD

**Second Editor's Comment:** Thanks to Drs. Williams and Sandberg for their comments. The Minto article raises so many important questions and they add two additional issues: (1) should surgery be avoided where possible and (2) how useful are "gender teams"? Clearly, methods and approaches, both surgical and psychological, have changed over the years. Those working in the field will continue to collect experience and share their insights through collaborations and multi-center trials. In an era of "the informed consumer", there does not yet seem to be a perfect approach—but as much information and openness as possible will help parents, families, and affected individuals make decisions that seem right for them.

Judith G. Hall, OC, MD

## ABSTRACTS FROM THE LITERATURE

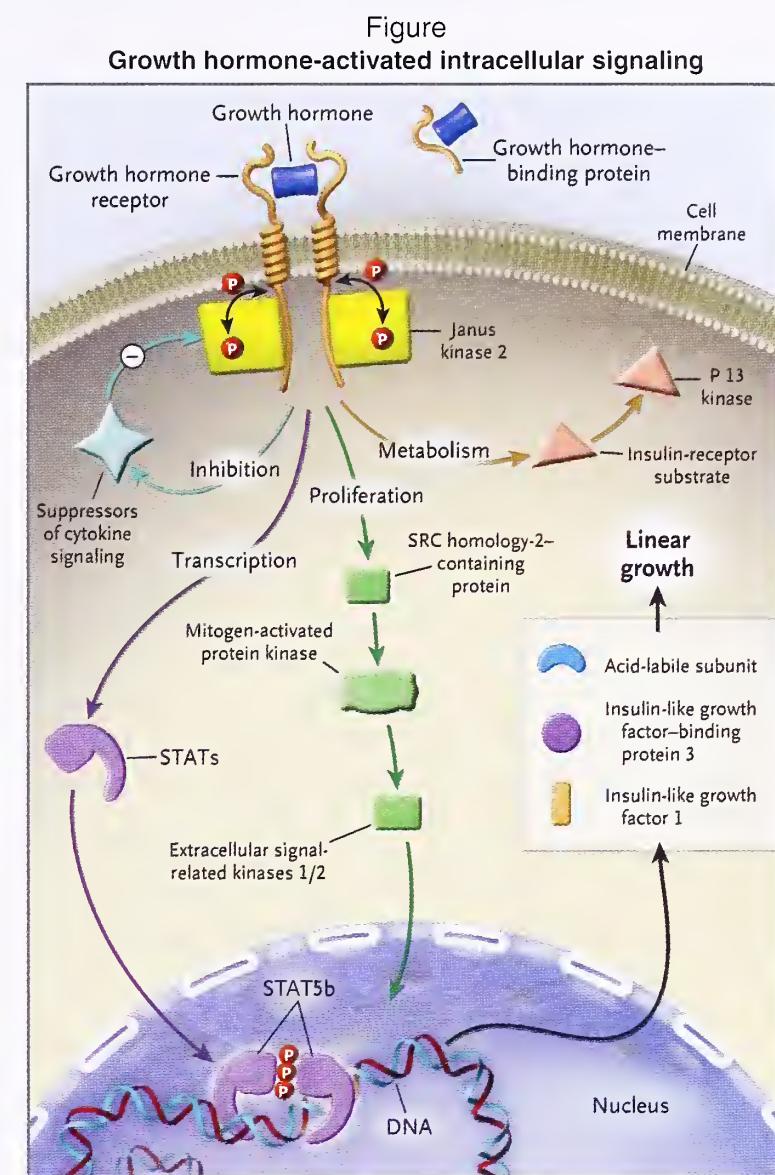
## STATs Role in Growth Hormone Insensitivity

Kofoed and colleagues described a patient with a homozygous mutation in the gene for STAT5b, resulting in growth hormone insensitivity. The girl had abnormal postnatal growth, facial dysmorphism, elevated GH levels after insulin-arginine stimulation, and markedly reduced serum concentrations of IGF-1, IGFBP-3 and acid labile-subunit. Serum concentrations of these proteins remained abnormally low despite 7 days of treatment with GH, and the growth rate failed to increase in response to one year of treatment. Concentrations of GHBP were normal, reflecting the fact that her GHR gene and protein were normal. This patient had no family history of growth retardation, though the parents were first cousins.

Kofoed EM, Hwa V, Little B, et al. Growth Hormone Insensitivity Associated with *STAT5b* Mutation. *N Engl J Med* 2003;349:1139-1147.

**Editor's Comment:** The authors described a novel mechanism for impaired growth in a patient with severe short stature. This was clearly demonstrated by sophisticated analysis. The finding of elevated serum GH levels after stimulatory tests, in conjunction with low levels of IGF-1 and IGFBP-3, established the presence of GH resistance. Unlike patients with the classic Laron syndrome, this patient had normal levels of GHBP, indicating that the defect was distal to the extra cellular GH receptor domain. A homozygous missense mutation in the *STAT5b* gene resulted in loss of GH action due to a post-receptor abnormality in the GH-signaling cascade. The GH-activated intracellular signaling involves several steps and, theoretically, each one of these steps could fail and produce GH insensitivity. The role of STATs in stature was reviewed in an accompanying editorial.<sup>1</sup> Eugster and Pescovitz depicted the GH-activated intracellular signaling cascade which is reproduced here. (Figure).

Fima Lifshitz, MD



Phosphorylation of the growth hormone receptor is followed by the activation of the metabolic, proliferative, and transcriptional pathways. STAT5b stimulates the transcription of factors (shown in the box) that are critical for normal linear growth. P denotes phosphorylation, and STAT signal transducer and activator of transcription.

Reprinted with permission from Eugster EA, Pescovitz OH. New revelations about the role of STATs in short stature. *N Engl J Med* 2003;349:1110-1112. Copyright © 2003 Massachusetts Medical Society. All rights reserved

## Reference

1. Eugster EA, Pescovitz OH. *N Engl J Med* 2003;349:1110-1112.

## IGF-1 Receptor Mutations in Intrauterine Growth Retardation

In a cohort of 41 children with intrauterine growth retardation (IUGR) and sustained postnatal growth retardation, the investigators detected 1 female subject (birth weight —3.5 SDS; adult height —4.5 SDS; exaggerated spontaneous and stimulated growth hormone [GH] secretion; normal to elevated IGF-1 concentrations; adult height apparently unresponsive to therapy with GH) who was a compound heterozygote for loss-of-function missense mutations in the gene encoding

the receptor for insulin-like growth factor I (*IGF1R*). Both parents were heterozygous for different mutations in exon 2 of *IGF1R* (mother, Lys115Asp; father, Arg108Gln). The birth weights and adult heights of both parents were modestly impaired (mother's birth weight —2.0 SDS; adult height —0.6 SDS; father's birth weight —2.0 SDS; adult height —2.8 SDS). The mutations in exon 2 result in IGF-1 binding and decreased sensitivity to this growth factor, hence limiting intracellular signal transduction.

A heterozygous nonsense mutation (exon 2: Arg59Stop) resulting in a truncated product was identified in 1 (male) of 9 children studied with short stature (height SDS —3.8 SDS at chronologic age 14 months) and elevated serum concentrations of IGF-1 who also had IUGR (birth weight —3.0 SDS, birth length —4.6 SDS). A similar mutation was present in the mother (birth weight —2.4 SDS; adult height —2.6 SDS) and brother. The mutation led to decreased numbers of *IGF1R* expressed on the plasma membrane of the patient's cultured fibroblasts and presumably to decreased sensitivity to ligand. The authors conclude that inactivating mutations of *IGF1R* are present in a small number of children with both IUGR and postnatal growth retardation, particularly in those with an elevated serum concentration of IGF-1.

Abuzzahab MJ, Schneider A, Goddard A, et al. IGF-1 receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med.* 2003;349:2211-2222.

**First Editor's Comment:** This report documents another abnormality leading to GH non-responsive growth retardation—this in the gene encoding the IGF-1 receptor. There are now documented loss-of-function mutations in the genes encoding the receptor for GH-releasing hormone, GH, the GH receptor, an essential protein (STAT5) in the signal transduction system for GH, IGF-1, and the IGF-1 receptor; abnormalities in the GHRHR and IGF1R signal transduction systems likely exist as well. An interesting perspective by Rosenfeld<sup>1</sup> discussing factors that control growth accompanies this article.

Allen W. Root, MD

**Second Editor's Comment:** Abuzzahab and colleagues described 2 patients with IUGR and sustained post-natal growth failure due to *IGF1R* gene mutations. In a related paper published the same month, Okubo et al<sup>2</sup> described a girl with IUGR and sustained postnatal growth failure through 10 years of age, despite GH therapy due to a de novo terminal deletion of chromosome 15q26.1, which led to a single gene copy of *IGF1R*. In vitro studies with cultured fibroblasts from skin biopsy revealed: decreased cell proliferation in response to IGF-1, a reduced IGF-1-stimulated *IGF1R* tyrosine phosphorylation, and decreased [<sup>125</sup>I]IGF-1 binding sites per cell but normal IGF-1 binding affinity. The girl also had facial and musculoskeletal dysmorphisms, a single café-au-lait spot, cardiac anomalies (atrial septal defect and ventricular septal defect), and developmental delays with learning difficulties. Her chromosomal deletion was cytogenetically visible; thus, the girl's phenotype may be due, in part, to contiguous gene deletions beyond the *IGF1R*.

As an elegant counterpoint, Okubo and colleagues also described a boy with 3 copies of the *IGF1R* gene due to chromosomal translocation who had dysmorphic features and was large from birth on.<sup>2</sup> Thus, alterations in the *IGF1R* gene—either mutations or abnormal gene copy numbers—may significantly affect growth, both prenatally and postnatally.

Adda Grimberg, MD

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2. Okubo Y, et al. *J Clin Endocrinol Metab.* 2003;88:5981-5988.

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## Overlap Between Schmid Metaphyseal Chondrodysplasia and Cartilage-Hair Hypoplasia

Schmid metaphyseal chondrodysplasia (MCD) is characterized by short stature, bowed legs, coxa vara, metaphyseal changes on skeletal X-rays, and autosomal dominant inheritance. It is usually considered to be easily distinguished from the autosomal recessive cartilage-hair hypoplasia (CHH), in which the skeletal findings are typically more severe and frequently accompanied by hair abnormalities, defective immunity, and hematologic disturbances. However, as Ridanpää and colleagues report, this may not always be the case. Although many patients with Schmid MCD have heterozygous mutations of the *COL10A1* (the gene encoding the type X collagen chain), some do not. In the study of 32 patients with a clinical diagnosis of Schmid MCD reported in this paper, *COL10A1* mutations were identified in 12 patients. Even though they lacked the non-skeletal features of CHH, the 20 patients with no *COL10A1* mutations were screened for mutations of the *RMRP* gene, which encodes the non-translated RNA component of the RNase mitochondrial

RNA processing complex (RNase MRP) and which is mutated in patients with CHH.

Two of the Schmid MCD patients (both 5-year-old boys), one of Canadian descent, the other of French-Canadian descent, were found to be homozygous for a base substitution G for A at nucleotide 70 of *RMRP*. This is considered a worldwide "major" mutation for CHH. In one case, parents were found to be heterozygous for this mutation consistent with the recessive inheritance of CHH. Review of the clinical findings confirmed that both boys had normal hair, no excessive ligamentous laxity, and normal history of infections with normal immunological and hematological findings. One was the product of a consanguineous mating.

The authors also searched for mutations in another gene *H1RNA*, which encodes the RNA component of RNase P, which is structurally and functionally similar to RNase MRP. No mutations were identified. The authors concluded that these patients represent the mild end of the clinical spectrum of CHH, and caution that it should be considered in patients with clinical features of Schmid MCD in whom a *COL10A1* mutation can not be found, especially if there is no family history for bone dysplasia.

Ridanpää M, Ward LM, Rockas S, et al. Genetic changes in the RNA components of RNase MRP and RNase P in Schmid metaphyseal chondrodysplasia. *J Med Genet*. 2003;40:741-746.

**Editor's Comment:** This paper provides another example of disorders that resemble each other clinically but have different genetic origins. It also underscores why identification of causative mutations is important. As the authors note, it is unknown if mild cases of CHH, such as those reported here, carry the same risk for complications such as skin and lymphoid cancers as more severely affected CHH patients. However, they may well since they carry the same *RMRP* mutation making surveillance for such complications an essential component of their care.

William A. Horton, MD

**Table**  
Clinical features in Schmid type of metaphyseal chondrodysplasia (MCDS) and cartilage-hair hypoplasia (CHH)

Feature	MCDS	CHH
Neonatal onset	—	++
Progressive	++	++
Disproportionate (short limbs)	++	++
Adult height	130-145 cm	110-140 cm
Bow legs	++	+
Increased joint laxity	—	+
Sparse hair	—	+
Immunodeficiency	—	+
Anaemia and marcocytosis	—	+
Increased risk of malignancy	—	+
Hirschsprung disease	—	+
Mode of inheritance	Autosomal dominant	Autosomal recessive
Responsible gene	<i>COL10A1</i>	<i>RMRP</i>

++, always present; +, often present; -, absent.

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## C-type Natriuretic Peptide and Achondroplasia

C-type natriuretic peptide (CNP) is a member of a family of 3 related peptides—atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and CNP. They act by inducing accumulation of intracellular cGMP through 2 subtypes of guanylyl cyclase: guanylyl cyclase A for ANP and BNP and guanylyl cyclase B for CNP. Although the natriuretic peptides are known mainly for regulating the cardiovascular system, there is growing evidence that CNP is an important positive regulator of endochondral bone growth. For example, genetically engineered mice have short bones when null for CNP and long bones when CNP is overexpressed. In fact, growth plates in these mice are shortened and widened in a manner similar to that detected in mice with loss- and gain-of-function mutations for FGFR3, respectively. These observations led the group headed by Nakao to propose a functional relationship between CNP and FGF signaling in the growth plate, which they have now demonstrated by mouse genetics.

The group first generated transgenic mice in which CNP was overexpressed in the growth plate; expression of the gene encoding CNP, designated *Nppc*, was driven by the type II collagen cartilage-specific promoter (*Col2*). The *Col2-Nppc* transgenic mice displayed excessive skeletal growth that was mainly postnatal. Compared to non-transgenic littermates, the *Col2-Nppc* transgenic mice had longer body length, longer limb bones, a longer cranial base (measured as naso-occipital distance), and wider growth plates by histology.

Next, the *Col2-Nppc* transgenic mice were mated to another transgenic mouse strain in which the achondroplasia-activating mutation of FGFR3 was expressed in cartilage also under the control of the type II collagen promoter (*Col2-FGFR3<sup>ach</sup>*). The latter mouse strain exhibits a dwarf phenotypic with characteristics of human achondroplasia and is considered an animal model for this condition. Offspring of this mating that carried both the *Col2-Nppc* and *Col2-FGFR3<sup>ach</sup>* transgenes had near normal body lengths when measured over 10 weeks. At 3 months, measurements of cranial base length, femurs, and humeri were statistically the same as non-transgenic mice, indicating that over-expression of CNP in the growth plate had rescued the dwarfism caused by the achondroplasia transgene. There was also restoration of the shortened growth plate of the *Col2-FGFR3<sup>ach</sup>* mice toward normal in the mice harboring both transgenes. Of note, the over-expression of CNP did not appear to rescue the reduced proliferation of growth plate chondrocytes detected in the *Col2-FGFR3<sup>ach</sup>* mice.

To confirm the direct effect of CNP on bone growth, the authors treated cultured tibias from *Col2-FGFR3<sup>ach</sup>* mice with different doses of CNP. Bone length showed a dose response to the CNP. The dose that restored bone

length to normal also restored synthesis of 2 markers of cartilage matrix biosynthesis—glycosaminoglycan and collagen—which were reduced in the *Col2-FGFR3<sup>ach</sup>* mice to near normal.

The authors next examined the effect of CNP on FGFR3 signaling pathways in the tibial explants. No differences were observed in FGF-induced STAT1 signaling, which has been implicated in the control of chondrocyte proliferation. However, CNP reduced signaling through the MAP kinase-ERK pathway.

The model that Yasoda et al<sup>1</sup> constructed suggests that FGFR3 signals through STAT1 to down regulate chondrocyte proliferation and differentiation and through the MAP kinase-ERK pathway to negatively control matrix synthesis in the growth plate. They propose that CNP blocks the MAP kinase inhibitory signals of FGFR3 to increase matrix synthesis and thereby counters the restraining consequences of FGFR3 on bone growth. They speculate that these observations could form a basis for a new therapeutic approach to treating achondroplasia.

Yasoda A, Komatsu Y, Chusho H, et al. Overexpression of CNP in chondrocytes rescues achondroplasia through MAPK-dependent pathway. *Nat Med*. 2004;10:80-86.

**Editor's Comment:** This is a very interesting paper that brings to the fore a growth plate regulatory circuit that has not been widely appreciated in the bone growth field. It also suggests that contrary to the popular view that activating FGFR3 mutations acts primarily through inhibition of chondrocyte proliferation and differentiation, they may also act by inhibiting the synthesis of the extracellular matrix that also contributes to bone growth.

The idea that CNP could be used to stimulate growth in achondroplasia is intriguing. Obviously, this work needs to be confirmed and much more investigation done, but in theory, blocking a downstream pathway that propagates growth inhibitory FGFR3 signals has promise.

Of caution is that high levels of CNP likely generated in cartilage of the transgenic mice, which presumably would be needed to counter the effects of mutant FGFR3 in patients, may be very difficult to achieve in a therapeutic setting, especially without having adverse effects on other tissues that respond to CNP such as kidney, adrenal gland, and cardiovascular system or on other regulatory circuits that utilize MAP kinase-ERK pathways. Nevertheless, the unfolding of this story deserves considerable attention.

William A. Horton, MD

## TBX1 in del 22q11.2 Syndrome (Di George)

In mice, heterozygous and homozygous loss-of-function (LOF) mutations in *Tbx1* (the murine ortholog of a gene present in the DiGeorge critical region [DGCR] on human chromosome 22q11.2) resulted in anomalies of the cardiac outflow tract, thymic and parathyroid gland abnormalities, and craniofacial defects. Nevertheless, previous investigators have been unable to document mutations in *TBX1* in patients with a DiGeorge-like phenotype. The authors demonstrate that heterozygous LOF mutations in *TBX1* can be responsible for the major clinical manifestations of the DiGeorge (DGS-hypoparathyroidism, thymic dysfunction, and cardiovascular anomalies) and conotruncal anomaly face syndromes (CAFS). They present an extremely carefully clinically characterized cohort of 235 Japanese patients with either DGS or CAFS. Fluorescence in-situ hybridization with site specific probes (FISH) analysis revealed microdeletions in the DiGeorge critical region of chromosome 22q11.2 in 225 (96%) patients. In 3 patients

in whom no microdeletion of chromosome 22q11.2 could be detected despite examination with multiple probes, mutations in *TBX1* were identified: exon 4:443T6A leading to Phe148Tyr; exon 8: 928G6A leading to Gly310Ser; exon 9: 1223delC leading to a stop signal at codon 459 and truncated product. *TBX1* is a member of a family of T-box transcription factors important for specifying mesodermal differentiation. It is a 10-exon gene that is transcribed into 3 products (TBX1A, TBX1B, TBX1C); the first 2 mutations would affect all products while the third would alter only TBX1C. Clinical correlation revealed that those patients with the first 2 mutations had classical CAFS and DGS, respectively; the patient with the third mutation had a less severe form of CAFS. These observations suggest that the clinical manifestations of the DGS/CAFS depend in part upon the extent of *TBX1* loss.

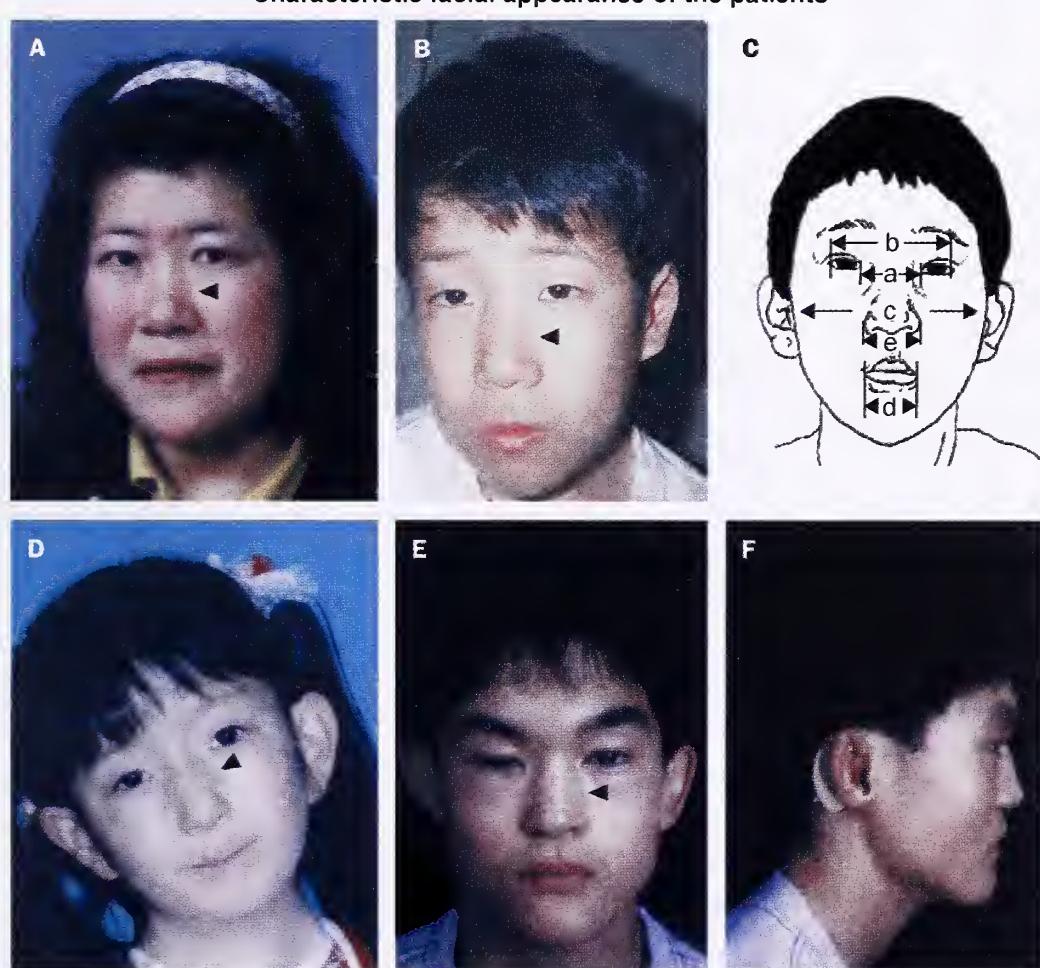
Yagi H, Furutani Y, Hamada H, et al. Role of *TBX1* in human del22q11.2 syndrome. *Lancet*. 2003;362:1366-1373.

**Editor's Comment:** This report not only identifies *TBX1* as an important determinant of DGS/CAFS, it also demonstrates the importance of a careful and complete clinical description of a complex and multidimensional clinical disorder. Twenty-five years of experience in treating patients with illnesses associated with microdeletions of chromosome 22q11.2 enabled these investigators to precisely define specific clinical criteria for the diagnoses of DGS/CAFS; they identified several abnormalities of the face that typified these patients (Figure). Thus, they were able to study patients who clearly had either DGS or CAFS. Therefore, LOF mutations in *TBX1* are associated with 5 phenotypes: abnormal face, velopharyngeal insufficiency, cardiac outflow anomalies, and thymic and parathyroid dysfunction. They are not associated with developmental delay often encountered in patients with DGS/CAFS, implying that another gene(s) in the DGCR is likely responsible for this problem. Mutations in *TBX1* were not detected in other subjects with DGS/CAFS, indicating there may be abnormalities in the non-coding region of this gene or that this disorder is polygenic in origin. In this regard, experimental deletion of *Fgf8* leads to a murine phenotype quite similar to that of the DGS/CAFS, suggesting that the products of *TBX1* may regulate the transcription of this gene. An interesting commentary accompanies this report.<sup>1</sup>

Allen W. Root, M.D.

### Reference

1. Baldini A. *Lancet*. 2003;362:1342-1343.



(A, B) Typical conotruncal anomaly face of del22q11.2 syndrome in familial cases (mother and son). Arrowheads show the area of the nose that seems to be divided into two parts (upper and lower) at the joint of the wing and at the sides. (C) Items of anthropometric measurement—a ÷ c (wide ocular hypertelorism), (b—a) ÷ b (short palpebral fissures), d ÷ c, and d ÷ e (small mouth). a=inner canthal distance. b=outer canthal distance. c=transverse facial width. d=oral width. e=nasal width. (D) Facial appearance of a patient with conotruncal anomaly face syndrome without the 22q11.2 deletion. (E, F) Facial appearance of a patient with DiGeorge's syndrome without the 22q11.2 deletion (E, frontal view; F, side view). Arrowheads show the area of the nose that seems to be divided into two parts (upper and lower) at the joint of the wing and at the sides.

Reproduced from *Lancet*, Yagi H, Furutani Y, Hamada H, et al. Role of *TBX1* in human del22q11.2 syndrome. *Lancet*. 2003;362:1366-1373.

## Criss-Crossing the Insulin and Insulin-like Growth Factor Pathways

Insulin and the insulin-like growth factor (IGF)-II both bind the A isoform of the insulin receptor (IR-A); how do the 2 hormones achieve specificity in responses? Pandini et al used microarray technology to test the hypothesis that the hormones affect different patterns of gene expression. They studied IGF-I receptor (IGF-1R)-deficient murine fibroblasts (R<sup>-</sup>cells) to isolate the effects on the IR from possible IGF-1R cross-reactivity. Some R<sup>-</sup> cells were transfected with human IR-A cDNA (R<sup>-</sup>/IR-A cells; ~ 5 X 10<sup>5</sup> IR's/cell) for comparison with the R<sup>-</sup> cells (~5 X 10<sup>3</sup> native IRs/cell), and gene expression profiles were compared following exposure to insulin or IGF-II. Two hundred fourteen transcripts were similarly regulated by the 2 hormones, and 45 were differentially transcribed. Expression patterns are summarized in the Table.

To validate the microarray data, changes in 12 genes belonging to different functional categories were confirmed by real-time PCR. Surprisingly, the different gene profiles of the 2 hormones did not fit neatly with the original functional dichotomy. For example, 3 genes selectively up-regulated by insulin were involved in regulating angiogenesis and differentiation, and 3 genes up-regulated longer after IGF-II than insulin were involved in metabolism (cholesterol metabolism, phosphate transport, and selenium supply/oxidative stress prevention). The authors concluded that these studies provided a molecular basis for the biological differences between insulin and IGF-II.

Pandini G, Medico E, Conte E, Sciacca L, Vigneri R, Belfiore A. Differential gene expression induced by insulin and insulin-like growth factor-II through the insulin receptor isoform. *A J Biol Chem.* 2003;278:42178-42189.

**Editors Comments:** The somatomedins were renamed the insulin-like growth factors (IGFs) for their primary structural homology to pro-insulin. Because of their different functions, the IGF and insulin systems were believed to be separate; IGF-I and IGF-II both stimulate cell survival and proliferation through the type 1 IGF receptor (IGF-1R), while insulin affects metabolism through the IR. The IGF-IIR, which is identical to the mannose 6-phosphate receptor, serves to clear IGF-II from the circulation.

Then things became more complicated. Not only do the ligands share structural homology, but the receptors are also very similar and they overlap in function. Both IGF-1R and IR are transmembrane  $\alpha_2\beta_2$  aggregates with autocatalytic tyrosine kinase activity, and both receptors

activate signaling cascades in common (MAP kinase pathway and PI3 kinase/Akt pathways). The IGFs and insulin can have similar metabolic effects; IGF-II over expression by tumors may cause hypoglycemia, and insulin is recognized as a growth-promoting hormone (best exemplified by the macrosomy of infants of diabetic mothers and babies with congenital hyperinsulinism).

Table

Differential gene expression patterns of IR-A detected by microarray technique

	Number of genes up-regulated	Number of genes down-regulated
By insulin alone	3	9
By IGF-II alone	5	1
Persistently changed by both hormones, more potently after IGF-II than insulin	5	3
Transiently changed by both hormones, longer after IGF-II than insulin	8	0

In addition to the IR found in metabolically responsive adult tissues—fat, liver, and muscle (the IR-B isoform)—there is also a shorter IR-A isoform (12 amino acids omitted from the  $\alpha$ -subunit by skipping exon 11). IR-A is the predominant isoform in fetal tissues and binds both insulin and IGF-II with high affinity.<sup>1</sup> IR-A is also over-expressed in cancers. Hybrid receptors, composed of an IR hemireceptor combined with an IGF-1R hemireceptor, have also been identified and implicated in neoplasia. These hybrid receptors are not just structural mistakes; they have been shown to bind IGF-I (but not insulin) with high affinity and contribute to IGF-stimulated cell growth.<sup>2</sup>

Thus, the question now is no longer, are the IGF and insulin systems truly related, but rather, how do they achieve specificity in response? One possible mechanism involves differential expression profiles of the various receptors that are cell-type- and ontogeny-dependent. Another involves differences in relative ligand concentrations, ligand binding affinities, and receptor densities. A third possibility evokes different downstream targets.

This paper is an important contribution to the field. Unfortunately, by providing a novel glimpse into the system, it makes it all seem a bit more complicated still.

Adda Grimberg, MD

### References

1. Frasca F, et al. *Mol Cell Biol.* 1999;19:3278-3288.
2. Vella V, et al. *Molec Pathol.* 2001;54:121-124.

## Klinefelter Syndrome: Phenotype and New Research

In August of 2000, the National Institutes of Health sponsored a meeting (co-sponsored by the March of Dimes and Klinefelter Syndrome and Associates) to address gaps in understanding this condition. In an effort to prioritize research initiatives, the participants summarized research data and developed consensus conclusions. Domains covered included: cytogenetic origin and molecular pathogenesis; gonadal and hormonal dysfunction; somatic anomalies; IQ and language development; adult-onset disorders; predicted phenotype and genetic counseling after *in utero* detection of XXY; genetic risks for offspring of the 47, XXY male; and, research priorities.

In contrast to autosomal trisomies in which maternal errors predominate (95% of cases), the origin of 47, XXY is far more variable with regard to source (maternal vs. paternal), as well as form of error (meiotic or mitotic origin). Adverse phenotypic outcomes are assumed to result from the action of excess genes on the X chromosomes that are not inactivated. Researchers are focusing attention on approximately 40 genes on the X short arm that escape inactivation.

Testicular histology in Klinefelter syndrome (KS) is normal or near normal in early infancy, and is followed by a progressive loss of germ cells throughout childhood. Animal models of sex chromosome aneuploidy have been developed to determine whether germ cell and Leydig cell defects are somatic or germ cell in origin.

Although testosterone concentrations fall within the normal range for 50% of late adolescent and young adults with KS, gonadotropins are universally elevated. Does this observation reflect compensated hypergonadotropic hypogonadism, or partial androgen resistance? Should testosterone levels in the normal range serve as the "gold standard" for guiding the timing and dosage of hormone replacement? The suggestion was made (but without supporting evidence) that prepubertal testosterone treatment (even in the early months of life) may normalize aspects of the behavioral phenotype in KS.

The relationship between hypogonadism and reduced libido in men is well documented, as is its effective treatment through testosterone replacement. Less well recognized and understood is the possibility of an increased prevalence in atypical psychosexual development (eg, paraphilic) among men with KS. Because the majority of individuals with KS go undiagnosed, ascertainment bias may be a factor in the association between sex chromosome aneuploidy and sexual disorders.

Neurodevelopmental studies reveal that XXY infants show decreased truncal tone and atypical gross motor skills with delays in walking (mean of 18 months) which can be ameliorated through intervention (mean of 12 months). IQ falls in the low normal range. Intellectual

functioning and career attainment are typically lower in boys with KS as compared with unaffected siblings. Language skills, in particular, are delayed with first words spoken between 18 to 24 months (vs. 12 months normally). These delays persist, and affect multiple aspects of language development. The characteristic passive personality, which is sometimes accompanied by paradoxical behavioral outbursts, may result out of frustration related to deficits in verbal skills under socially challenging circumstances.

Accumulating evidence indicates that XXY is associated with autoimmune disorders. The link in pathogenesis may be chronic estrogen stimulation. Breast cancer appears to be markedly increased in older XXY men, as are extragonadal germ cell cancers and mediastinal teratomas. The authors speculate that constitutional chromosomal abnormalities and extragonadal aneuploidy germ cells predispose to malignant degeneration.

Simpson JL, de la Cruz F, Swerdloff RS, et al. Klinefelter syndrome: expanding the phenotype and identifying new research directions. *Genet Med.* 2003;5:460-468.

**Editor's Comment:** Just when you thought you knew all you needed to know about KS, along comes a summary of a conference indicating that research continues unabated. When the diagnosis of KS is made during childhood or adolescence, the pediatric endocrinologist should be the lead healthcare professional involved with the child's care. In light of evidence that neurocognitive deficits associated with KS are apparent at an early age, a developmental assessment should be performed. A referral to a psychologist should be accompanied with background readings (such as this conference proceeding) to orient that clinician to the most current syndrome-specific findings. The objective here is to ameliorate the predictable deficits, thereby improving developmental and quality-of-life outcomes. Such interventions would ideally occur long before issues of testosterone replacement become the focus of clinical management.

The suggestion that early testosterone treatment may normalize socialization difficulties in boys with KS is intriguing. Although changes in clinical care await conclusive evidence of such benefits, the discussion forces us to think about testosterone acting in ways beyond induction of pubertal development.

Finally, recent findings regarding adult-onset disorders in KS provide the pediatric endocrinologist with the opportunity to emphasize the importance of regular visits with adult specialists.

David E. Sandberg, PhD

## Soy Formula Complicates the Management of Congenital Hypothyroidism

High fiber soy flour has been reported to cause goiter formation and hypothyroidism in humans and other animals. Soy-based formulas are now made from isolated soy protein to which iodine has been added. Whether or not these contemporary formulas could be associated with neonatal thyroid dysfunction was investigated by Conrad and colleagues in a retrospective analysis of patient charts at Children's Memorial Hospital in Chicago. Seventy-eight children born between 1990 and 1998 who were followed at CMH until at least 1 year of age were eligible for the study. Data included weight, length, total T4, TSH, levothyroxine dose, dietary information, and thyroid scan results. All children diagnosed with congenital hypothyroidism had a thyroid scan at their facility.

Eight children received soy formula, and 70 did not. Treatment was started 2 days earlier (median data) in the soy group. There were no significant differences between initial levothyroxine doses or the 1-year dose between the groups. T4 and TSH levels were comparable before the start of therapy, but TSH levels were significantly higher in the soy group at the first evaluation following initiation of therapy (42.6 mU/l vs 6.6 mU/l,  $p<0.01$ ). Time to normalization of TSH levels was significantly longer in the soy group (150 days vs. 40 days,  $p=0.02$ , median data). At 6 months, 62.5% of the soy group had elevated TSH values compared to 17% of the non-soy group ( $p=0.01$ ). The difference in TSH values persisted throughout the first year of life. There were no significant differences in

height or weight, z-scores, or any other parameter.

The authors discuss possible reasons for their findings, including severity of hypothyroidism, immaturity of the T4-TSH feedback loop, or inadequate dosing, none of which could be demonstrated in this study. The authors speculate that the cause is malabsorption and increased fecal loss of levothyroxine. Free T4 levels were not measured in these children. Neuropsychological data were not available.

Conrad S, Chui H, Silverman B. Soy formula complicates management of congenital hypothyroidism. *Arch Dis Child*. 2004; 89:37-40.

**Editor's Comment:** This retrospective study provides some provocative information. Although these infants had similar total T4 levels, thyroid dysfunction was present throughout the first year of life in those fed a soy-based formula. Whether or not there are any long-term sequelae related to this disparity remains to be seen. Newer recommendations for treatment of congenital hypothyroidism are based on normalization of TSH levels at a much quicker rate than previously. It would appear that infants fed soy-based formula will require higher doses of levothyroxine than those on other diets. This is important information for pediatricians and pediatric endocrinologists. A dietary history remains an important part of every child's health evaluation.

William L. Clarke, MD

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## OBESITY OF INFECTIOUS ORIGIN – A REVIEW

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### INTRODUCTION

Obesity has become the number one public health problem in America.<sup>1</sup> Obesity is a complex, multifactorial disease that involves the interaction of genetic, metabolic, social, behavioral and cultural factors. In the decade from 1980 to 1990, the number of people with obesity increased by 30% in the US; the number of obese adults further increased to 61% between 1991 and 2000.<sup>2</sup> The numerous health risks associated with obesity are well known to the medical community.

The epidemic increase in obesity, its medical consequences, and the rapidly escalating health care costs associated with it have prompted a multidisciplinary approach by health professionals, government, and non-governmental

### From The Editor's Desk

Obesity has reached epidemic proportions worldwide; the term "globesity" defines the current situation. If the prevalence of obesity remains unabated this will be the first generation of children who die before their parents! The disease is now attracting the attention of pediatric endocrinologists. At the LWPES/APS there were multiple presentations on the subject and a symposium on adiposity. Obesity is now recognized to be at the crossroads of insulin resistance, a condition implicated in the "deadly quartet" of western civilization: diabetes mellitus, hyperlipidemia, hypertension and cardiovascular disease, as well as other common pediatric endocrine conditions, ie, PCOS, acanthosis nigricans, glucose intolerance, etc. The obesity epidemic has its roots in a lifestyle which facilitates consumption of excess calories over and above energy expenditures.

Adipocytes function as an endocrine organ and play an important role in the pathogenesis of obesity and its complications. However the potential role of infectious agents triggering or being associated with obesity and/or its co-morbidities is rarely discussed, nor are the potential endocrine alterations that may be induced by infective processes. In this issue of *GGH* such an omission is addressed by Drs. Dhurandhar, Atkinson and Ahmed. Their review should shed light and attract attention to this poorly understood area and facilitate an understanding of obesity in its entirety. Filling the void of this often neglected aspect may also stimulate research by pediatric endocrinologists wishing to clarify the endocrine interactions with adipocytes and infective agents.

The editors have reviewed a variety of papers addressing subjects of great interest. Noteworthy in the growth field are the papers on the long-term mortality of recipients of pituitary derived growth hormone, the novel dysfunctional growth hormone variant, the growth hormone and IGF-I effects on longitudinal growth, and cancer risk. Also note the papers disproving the risk of type 1 diabetes mellitus with childhood vaccinations as well as those addressing new discoveries of leptin action and a novel treatment of osteogenesis imperfecta. I also want to highlight the 2 papers on intersex, intersexuality and sexual identity which denote the current state of treatment controversies.

I am also pleased to bring to your attention enhancements in the print and web-based journal with the addition of color figures and a more efficient search capability. Please keep us posted with your comments and suggestions so we may continue improving the journal.

Respectfully,  
Fima Lifshitz, MD

### Highlights In This Issue

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organizations to search for new methods to control it.<sup>3</sup> Such efforts are likely to be facilitated by a better understanding of the etiology of obesity. Sclafani<sup>4</sup> classified the etiology of animal obesity into 9 groups, including obesity of neural, endocrine, pharmacological, nutritional, environmental, seasonal, genetic, idiopathic, and viral origin. Of these factors, the viral etiology of obesity, a relatively recent discovery first noted in 1982,<sup>5</sup> has barely been studied. Over the past 20 years, 8 pathogens have been reported to cause obesity in animal models.<sup>5-11</sup> The relative contribution of these pathogens to human obesity is not yet clear. Considering the emerging reports addressing an infectious etiology of several other chronic diseases,<sup>12</sup> the contribution of certain infections to the etiology and pathogenesis of obesity need not be inconceivable. If shown to be relevant to humans, this relatively novel concept may be potentially important. An adequate understanding of such pathogens is needed for better management of obesity. A new perspective about the infectious etiology of this disease may initiate additional research in the field to assess the contribution of pathogens in human obesity and its co-morbidities and possibly to prevent or treat the obesity of infectious origin.

The known obesity-producing infective agents are listed in Table 1. There are 7 viral pathogens known to cause obesity in animal models, 4 are either known human pathogens or have been shown to be associated with human obesity. In addition *Chlamydia pneumoniae* has been associated with obesity in humans. In this paper we review the present knowledge in the field as this may be of importance to those who deal with patients and/or those who are interested in obesity.

**Table 1. Pathogens responsible for obesity**

Pathogen (Reference)	Animal model	Possible Mechanism(s)
Human adenovirus-36* (11,15,16)	Chickens, mice, non-human primates	Up-regulation of pre-adipocyte differentiation
Human adenovirus-37* (33)	Chickens	Unknown
SMAM-1 adenovirus* (8,9)	Chickens	Unknown
Borna-disease virus* (10,50,51)	Rats	Hypothalamic damage
Chlamydia pneumoniae* (68)	No animal model, associated with weight gain in humans	Unknown
Scrapie agent (76-79)	Mice	Hypothalamic-pituitary-adrenal axis damage
Canine Distemper virus (5)	Mice	Hypothalamic damage, reduced hypothalamic leptin receptor expression
Rous-Associated virus-7 (6,7)	Chickens	Reduced thyroid hormone levels

\* Human pathogens, and/or associated with human obesity.

## ADENOVIRUS AND OBESITY

### Human Adenovirus Type-36

In 2000 we reported that adenovirus type 36 (Ad-36) causes adiposity in animals.<sup>11</sup> Adenoviruses are naked

DNA viruses with icosahedral symmetry and a diameter of 65-80 nm. In humans, adenoviruses are frequently associated with acute upper respiratory tract infections, and may also cause enteritis and conjunctivitis. Adenoviral infections are transmitted via respiratory, fomite, droplet, venereal, and fecal-oral routes; these are easily isolated from nasal swabs or from feces. There are more than 50 types of human adenoviruses listed with the American Type Culture Collection. Ad-36 cross-reacts minimally, or not at all, with other human adenoviruses<sup>13,14</sup> and apparently is antigenically unique. Ad-36 was first isolated in 1978 in Germany in the feces of a 6-year-old girl suffering from diabetes mellitus and enteritis.<sup>14</sup>

In 4 separate experiments, chickens and mice were inoculated with human adenovirus Ad-36.<sup>11</sup> These animals developed a syndrome of increased adipose tissue and paradoxically low levels of serum cholesterol and triglycerides. This syndrome was not present in chickens inoculated with avian adenovirus chick embryo lethal orphan virus (CELO).<sup>11</sup> Sections of the brain and hypothalamus of Ad-36 inoculated animals did not show any overt histopathological changes. Ad-36 DNA was detected in the adipose tissue, but not in skeletal muscles for as long as 16 weeks after Ad-36 inoculation. Subsequently, to ascertain if blood transfusion from Ad-36 infected chickens could produce adiposity in uninfected animals, 4 age- and weight-matched groups of chickens were used: infected donors and recipients (I-D, I-R) and control donors and recipients (C-D, C-R).<sup>15</sup> Blood was taken from the I-D and C-D groups and injected into the recipient groups. The I-D and the I-R groups developed 2.5 and 1.8 times more visceral fat as compared with the C-D group. Ad-36 DNA was detected in the adipose tissues of I-D and I-R groups, but not in the controls. The 2 infected groups showed significantly decreased serum cholesterol levels and the I-D group had a significant reduction in serum triglycerides. These data confirmed that Ad-36 produces adiposity and paradoxical reductions in serum lipids. In addition, the study fulfilled a Koch's postulate, namely that adiposity was transmitted from infected animals (I-D group) to a new set of animals (I-R group).

Furthermore, two studies were conducted in nonhuman primates to investigate the adiposity-promoting potential of Ad-36.<sup>16</sup> In the first study, spontaneously occurring

Ad-36 antibodies were detected in stored serum samples from adult male rhesus monkeys that were collected over a 7-year period at the Regional Primate Research Center located at the University of Wisconsin, Madison, WI. The monkeys gained approximately 0.1 kg of body

weight during the year preceding seroconversion, and gained 1.8 kg of weight during the following year. Serum cholesterol fell about 35 mg/dL after the appearance of Ad-36 antibodies. In the second experiment, male marmosets inoculated with Ad-36 had a 4-fold weight gain, with a 60% increase in body fat, and a 34mg/dL reduction in serum cholesterol levels as compared with controls over a 6-month period. These data demonstrate that Ad-36 is capable of increasing body fat in non-human primates.

### Mechanism of Action

The exact mechanism of action on adipocytes by Ad-36 is incompletely understood (Figure 1). Ad-36 was recently reported to up-regulate preadipocyte differentiation in-vitro.<sup>17,18</sup> Inoculation of 3T3-L1 preadipocytes with Ad-36, but not Ad-2, a non-adipogenic human adenovirus, resulted in increased adipocyte number, cellular lipid accumulation and glycerol 3-phosphate dehydrogenase levels (an adipocyte differentiation specific enzyme marker).<sup>17,18</sup> On the other hand, expression and secretion of leptin (an adipocytokine involved in body weight regulation) by Ad-36 inoculated fat cells was reduced compared to uninfected controls.<sup>19</sup> The phenomenon of increased lipid accumulation and decreased leptin secretion was observed in 3T3-L1 preadipocytes inoculated with Ad-36 or Ad-37, but not in Ad-2 inoculated cells.<sup>20</sup> Extrapolation of these findings to an *in-vivo* situation would suggest increased adipogenesis due to a relative absence of leptin. Thus, the mechanism may involve up-regulation of fat cell differentiation due to a local, direct effect of the virus, as well as a systemic effect of leptin.<sup>21</sup> The interaction of the viral and the cellular genes involved has not yet been elucidated.

### Adipose Tissue-Immune System Interaction

In light of well documented interactions of adipose tissue involvement with modulators and mediators of immune response, an adipogenic effect of certain pathogens should not be surprising. Cousin et al<sup>22</sup> reported that preadipocytes function like macrophages and possess phagocytic and microbicidal activity. Adipocytes too, participate in the immune response. Leptin, an adipocytokine, enhances proliferation and activation of human circulating T lymphocytes and stimulates cytokine production.<sup>23</sup> In addition to leptin-induced modulation of cytokine release, adipocytes themselves secrete various cytokines<sup>24,25</sup> and, in turn, preadipocytes and adipocytes are subject to cytokine directed modulations.<sup>26,27</sup> Certain cytokines, such as tumor necrosis factor alpha (TNF- $\alpha$ ), down-regulate preadipocyte differentiation<sup>27,28</sup> and increase leptin secretion by adipocytes<sup>30</sup> and adenoviral proteins sensitize cells to TNF  $\alpha$ .<sup>31</sup> Although Ad-36 reduces leptin expression and secretion from fat cells,<sup>19</sup> its effect on TNF  $\alpha$  is unknown. It is hypothesized, but not tested that Ad-36 proteins decrease both TNF  $\alpha$  levels and leptin, thereby contributing to up-regulation of preadipocyte differentiation by their relative absence.

Considering the extensive interaction between the immune system and the adipose tissue, expansion of the latter in response to certain infections is conceivable. For instance, Macrophage colony-stimulating factor, which promotes the production of macrophages, is also secreted by adipocytes and, when overexpressed *in vivo*, induces significant adipose tissue hyperplasia.<sup>32</sup> It is unknown if any of the obesity promoting pathogens stimulates macrophage colony-stimulating factor production leading to the growth of adipose tissue.

### Human Adenovirus Type-37

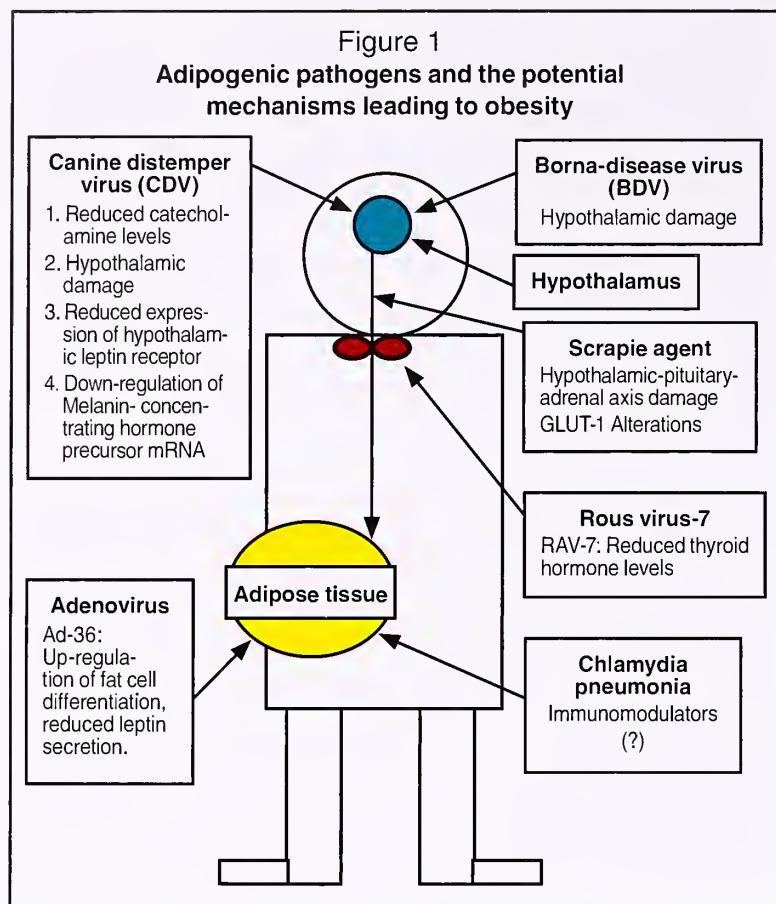
There are other adenoviruses with adipogenic potential properties. In preliminary studies it was demonstrated that Ad-37 increased adiposity in chickens, but that Ad-2 and Ad-31 did not.<sup>33</sup> Currently, minimal additional information is available on Ad-37, but the adipogenic mechanism of this virus may be similar to Ad-36 (Figure 1). However, these results demonstrate that more than one human adenovirus is capable of producing obesity in an animal model, but the adipogenic property is not necessarily shared by all human adenoviruses.

### Adenovirus and Human Obesity

In preliminary studies, human serum samples were obtained from over 500 obese (BMI  $\geq 30$  kg/M<sup>2</sup>) and non-obese volunteers from 3 different sites (Wisconsin, Florida, and New York). The sera were screened for the presence of Ad-36 antibodies using serum neutralization assays. A positive antibody status is suggestive of previous exposure of the individual to the virus. The prevalence of Ad-36 antibodies pooled across the 3 experimental sites was 11% for the non-obese and 30% for the obese subjects.<sup>34,35</sup> Antibody-positive subjects had a significantly higher BMI than antibody-negative individuals. Also, antibody-positive obese subjects had significantly lower serum cholesterol levels compared with the antibody-negative individuals.<sup>34,35</sup> Serum triglyceride measurements were only available at the Wisconsin site, the levels were significantly lower in the antibody-positive subjects versus the antibody-negative counterparts. These data demonstrated that antibody-positive humans were heavier and had lower serum cholesterol and triglycerides levels; these findings were similar to the data of experimentally infected animals with Ad-36. However, extensive research will be needed to establish the contribution of Ad-36 to the etiology of human obesity.

### Adenoviral Infections and Weight Gain in Children: Conjectures

It is well known that respiratory viral infections including adenoviral infections are very common among children.<sup>36,37</sup> Additionally, a very high prevalence of adenovirus is reported in lymphoid tissue obtained by tonsillectomy.<sup>38</sup> Although adipogenic properties of all adenoviruses have not been examined, it is interesting to note that excess weight gain occurs with or without



gain in height in children undergoing tonsillectomy.<sup>39-41</sup> It is not known if tonsillectomy provides the impetus for latent adenoviruses to promote the weight gain. In addition, obese and overweight children have higher levels of markers of inflammation.<sup>42</sup> It is now believed that excess body weight is associated with a state of chronic low-grade inflammation in children as measured by higher levels of C-reactive protein.<sup>43</sup> It is unknown if the inflammatory process is due to infections, or whether it is a causative factor for weight gain in children. Duncan and colleagues<sup>44</sup> showed that fibrinogen and other putative markers of inflammation can predict weight gain in middle-aged adults, which suggests a possible contribution of inflammation to weight gain and/or to co-morbidities associated with obesity. Longitudinal studies that track weight changes in children with and without adenovirus infections are needed to address these issues.

### SMAM-1 Avian Adenovirus

SMAM-1, an avian adenovirus identified in the early 1980s during a poultry epidemic,<sup>45</sup> was found to produce adiposity in chickens.<sup>8,9</sup> We inoculated 3-week-old chickens with SMAM-1 and noted development of excessive visceral fat and paradoxically lower levels of serum lipids compared to the uninfected controls.<sup>8,9</sup> Uninoculated chickens sharing the same room with inoculated chickens (in-contact group) developed the obesity syndrome, presumably due to infection with virus particles carried in the air.<sup>8,9</sup> There was no difference in food intake among the controls, inoculated, and the in-contact group. Visceral fat was greater by 53% and 33% in the inoculated and in-contact groups, respectively. SMAM-1 was reported to be associated with human

obesity. Antibodies to SMAM-1 were found in 11 of 52 subjects.<sup>46</sup> Antibody-positive subjects were heavier ( $95.1 + 2.1$  kg vs  $80.1 + 0.6$  kg,  $p < 0.02$ ) and had a higher BMI ( $35.3 + 1.5$  kg/m<sup>2</sup>, vs  $30.7 + 0.6$  kg/m<sup>2</sup>,  $p < 0.001$ ) vs the antibody-negative group. Serum cholesterol was 15% lower and triglycerides were 60% lower in SMAM-1 antibody-positive subjects. Since the prevailing thought was that avian adenoviruses do not infect humans and that human adenoviruses do not cross-react with avian adenoviruses,<sup>47</sup> the findings were surprising. It is possible that a human adenovirus antigenically similar to SMAM-1 produced antibodies that cross-reacted with SMAM-1. Further research is necessary to determine if SMAM-1 is capable of producing obesity and changes in serum lipids in humans. The potential mechanisms whereby infections with this virus lead to obesity remain to be proven (Figure 1).

### Borna Disease Virus

Borna disease virus (BDV) has also been implicated in obesity. This virus was first described in the early 1800s.<sup>48</sup> BDV, has been recently characterized as an enveloped, nonsegmented, negative-stranded RNA virus with a genomic size of approximately 9 kb and nuclear site for replication and transcription.<sup>49-51</sup> The genomic organization is similar to that of members of the Mononegavirales order; therefore, BDV is the prototype of the new family Bornaviridae within this order. BDV infects a broad range of warm-blooded animals from birds to primates. It replicates at lower levels than most known viruses,<sup>52,53</sup> is not lytic, and persists in the nervous system despite a vigorous immune response. Infected animals exhibit movement and behavior disorders.<sup>54,55</sup> BDV-specific antibodies were detected in asymptomatic horses in several countries,<sup>56-60</sup> suggesting that natural infections in animals remain subclinical in most cases.

Gosztonyi and Ludwig<sup>10</sup> reported that BDV infection produces a syndrome of obesity in rats, characterized by lympho-monocytic inflammation of the hypothalamus, hyperplasia of pancreatic islets, and elevated serum glucose and triglyceride levels. The expression of BDV-induced obesity syndrome varies with the age of the animals at the time of inoculation, the genetic background of the host and the viral strain used.<sup>10</sup> Rats infected as newborns with BDV show progressive neurological disease. On the other hand, weanling or adult rats similarly inoculated with BDV develop acute encephalitis and die within 1 to 4 months. Some of these rats survive the infection and develop marked obesity.<sup>61</sup> The obese phenotype has a characteristic distribution of inflammatory lesions and BDV-antigen in the rat brain. Infiltration with mononuclear immune cells and viral antigen expression are restricted to the septum, hippocampus, amygdala and ventromedial tuberal hypothalamus. Therefore, infection with obesity-inducing BDV most likely results in neuroendocrine dysregulations leading to development of obesity.<sup>62</sup> This might be due to the restriction of viral

antigen expression and inflammatory lesions to brain areas that are involved in the regulation of body weight and food intake (Figure 1).<sup>62</sup>

BDV may also be a human pathogen.<sup>48</sup> BDV-specific antigen and BDV-RNA were detected in 4 autopsied human brains with hippocampal sclerosis and astrocytosis. BDV-seropositive neurologic patients have been observed to become ill with lymphocytic meningoencephalitis.<sup>63</sup> In humans BDV is also associated with schizophrenia and mental depression<sup>64,65</sup> that are responsive to treatment by amantadine, an antiviral agent.<sup>66,67</sup> However, the contribution of BVD infections and the relationship to obesity in humans is unknown. Although it would be interesting to know if those with such infections gain more weight; such a relationship has not been reported.

### Chlamydia pneumoniae

The relationship between *Chlamydia (C) pneumoniae* infection and coronary heart disease (CHD) is of interest. There are studies that showed that *C. pneumoniae* was related to the development of CHD.<sup>68</sup> While others have found negative results,<sup>72-74</sup> in Australia newly identified cases of CHD compared with matched controls were tested for the presence of serum IgG and IgM against *C. pneumoniae*, *C. trachomatis* and *C. Psittaci*. None of the subjects had IgM against chlamydia and only few were positive for *C. trachomatis* and/or *C. psittaci*.<sup>73</sup> The prevalence of seropositivity for *C. pneumoniae* was not significantly different for subjects with or without CHD. Similarly, a number of known CHD risk factors such as hypertension, serum lipids, and glucose levels lacked a significant difference between the antibody-positive and antibody-negative groups. However the antibody-positive group had significantly greater BMI and smaller LDL particle size. Antibody prevalence was significantly greater for subjects with insulin levels above the median and for those with LDL particle size below the median. However, after multivariate analysis, only BMI continued to be associated with seropositivity.

Although the association of *C. pneumoniae* antibodies with CHD may be questioned, the increased BMI with seropositivity to this infection is very intriguing. Approximately 10% of the subjects were obese. The greater prevalence of antibodies in patients in the highest BMI quartile as well as the relationship of BMI with the presence of positive *C. pneumoniae* antibodies may be the result of impaired immunity. Unlike *C. pneumoniae*, antibodies to *C. trachomatis* and *C. psittaci* did not show such a selective or high prevalence among those with higher BMI. A possible explanation offered by Dart et al,<sup>68</sup> which has neither been proved nor disproved, is that *C. pneumoniae* infection may be causally related to increased BMI, though the mechanism involved in this process is not completely known.

### Scrapie Agent

Scrapie is a neurodegenerative disease of prion proteins, with a long incubation period, known to occur in sheep and goats. Scrapie affects the brain and is transmissible from animal to animal. The key features of such infections include abnormal behavior and deficits in motor function. Certain scrapie strains induce obesity in experimental animals.<sup>70,71</sup> The obesity-promoting characteristic is a function of the scrapie strain, but not the mouse type. Regardless of the mouse strain tested, scrapie strain ME7 induced obesity. The effect was not observed with scrapie strains 139A or 22L in mice.<sup>78</sup> Vacuolation in the forebrain of the mouse was caused by ME7, whereas 22L and 139A caused vacuolation in the cerebellum and white matter, respectively.<sup>77</sup> The difference in the obesity-promoting potential of the agents may be linked to the differences in the brain lesions. Kim et al<sup>79</sup> demonstrated that ME7-induced weight gain in mice was associated with increased adrenal gland weight and adrenalectomy prevented ME7-induced obesity. Based on these findings, they suggested that scrapie-induced obesity depends on an effect of scrapie on the hypothalamic–pituitary–adrenal axis (Figure 1). Recently, Vorbrot et al<sup>80</sup> demonstrated differences in the distribution of glucose transporter (GLUT-1) in the microvascular endothelium of scrapie-infected SJL/L hyperglycemic mice. These animals showed clinical signs of scrapie, obesity, and reduced glucose tolerance. GLUT-1 receptor density was significantly lower in microvasculature supplying the thalamus, cerebellum and, to a lesser degree, the hippocampus, but was unaffected in microvessels supplying the cerebral cortex and olfactory bulb.<sup>80</sup> Glucose, the major energy source for the brain, is passed across the blood–brain barrier by facilitative diffusion catalyzed by GLUT-1. Reduced GLUT-1 density in the scrapie-infected mice impairs transvascular glucose transport in the above-mentioned brain regions and presumably disturbs their function, which may lead to obesity.<sup>79</sup>

### Canine Distemper Virus

Canine distemper virus (CDV) was reported to cause obesity in mice in 1982.<sup>5</sup> CDV is a member of the genus Morbillivirus of the family Paramyxoviridae that causes severe health problems including respiratory, gastrointestinal, and central nervous system disease in dogs and other wild mammals.<sup>81</sup> CDV-induced encephalomyelitis in dogs is the most common cause of death.<sup>82</sup> CDV invades the nervous system and replicates in neurons and glial cells of the white matter during a period of severe viral-induced immunosuppression.<sup>83</sup> An increase in body weight and fat cell size and number was reported in Swiss albino mice experimentally infected with canine distemper virus.<sup>5</sup> Six to 20 weeks after CDV infection obesity was observed in approximately 26% of the mice with intracerebral infection compared to 16% of mice with intraperitoneal infection. Catecholamine levels were reduced significantly in the infected obese mice. The phenomenon of CDV-induced obesity in mice is believed to be due to virus-induced

hypothalamic damage.<sup>84-86</sup> Bernard et al<sup>87</sup> reported down-regulation of expression of the leptin receptor in the hypothalamus of CDV infected obese mice, and suggested this as the cause of the weight gain. Recently Verlaeten et al<sup>88</sup> demonstrated that melanin-concentrating hormone precursor mRNA, an anorexigenic neuromodulator was down-regulated in the late stage of acute phase of CDV infection in mice. Bernard et al<sup>87</sup> speculated that the data demonstrated a "hit and run" type of relationship between CDV and the expression of obesity, ie, the initial viral impact in the hypothalamus may initiate changes that would continue to promote obesity in animals even after the acute infection subsided. CDV is not considered a human pathogen, and its contribution to human obesity is unknown. However, measles virus is a human virus closely related to the CDV, and both belong to the paramyxovirus family, though its relationship to human obesity is not known. Animal experiments showing the effect of measles virus on adiposity are also unavailable.

### Rous Associated virus 7

Carter et al<sup>6</sup> reported that Rous-associated virus 7 (RAV-7) induced obesity in chicken characterized by stunting, hyperlipidemia, and hypercholesterolemia. Inoculation of 10-day-old chick embryos with RAV-7 produced fat deposition around crop and abdominal fat pads in the adult birds.<sup>6</sup> Intravenous inoculation of 1-day-old chickens with RAV-7 did not produce stunting and obesity.

Chicken embryos infected with RAV-7 developed fatty, yellow colored livers, hepatomegaly, anemia, and immune suppression.<sup>6</sup> Livers of infected animals constituted 6.2% of the body weight vs 2.4% of the body weight in the uninfected controls. These signs and symptoms manifested within 3 to 4 weeks after hatching. Obesity, stunting of growth and hyperlipidemia were the most striking features observed in the RAV-7 infected chickens. The mean body weight of the 50-day-old RAV-7 infected chickens was 515 g compared to 194 g of the same age controls. Both the RAV-7 infected and control groups were offered the same amount of food. Although the usual triglycerides levels for chickens are around 100 mg/dL, chickens from the RAV-7 group had serum triglycerides levels over 2000 mg/dL. The authors suggested that the reduced thyroid hormone level in the RAV-7 infected chickens was the cause of the observed obesity and hyperlipidemia.<sup>6</sup> Although lymphoblastoid infiltration of the thyroid gland was noted in the RAV-7 infected chickens, antibodies to thyroglobulin indicative of autoimmune thyroiditis, were absent. Administration of exogenous thyroxine prevented the syndrome.

### CONCLUSIONS AND SPECULATION

Although obesity has multiple causes, an overlooked possibility is that in some instances obesity could be due to an infection. Seven viral pathogens are reported to

cause obesity in animals. Of which, at least 4 are human pathogens and are associated with human obesity. In addition *Chlamydia pneumoniae* has also been associated to human obesity; however more research is needed to further define the mechanisms and the role of these pathogens in its etiology and/or co-morbidities.

It is possible that viral infections exacerbate and facilitate the development of obesity, or its complications, by working in conjunction with other adipogenic factors. For example obese children have been shown to have a cluster of conditions that put them at a high risk for developing diabetes and heart disease.<sup>89</sup> Over one-third of obese children studied presented with dysmetabolic syndrome, defined as hypertension, low HDL cholesterol, high insulin levels, elevated blood glucose and triglyceride levels. In addition, they presented elevated levels of C-reactive protein (CRP); which reflect an inflammatory reaction associated with an increased risk of heart disease. Furthermore, there were decreased levels of adiponectine with increased adiposity. Adiponectine is an anti-inflammatory hormone produced in fat cells that helps regulate glucose and cholesterol metabolism and may help protect blood vessels.

The insidious onset of human obesity makes it difficult to retrospectively link obesity or any of its co-morbidities to a particular episode of infection. Thus, a causative role for infectious pathogens in human obesity is difficult to establish. Due to ethical considerations, humans cannot be experimentally infected with these pathogens; linking the infection to long term weight gain is often impossible. In order to determine the role for viral pathogens in human obesity it is necessary to collect overwhelming indirect evidence in the area, and that remains to be done.

Elucidating the role of obesity of infectious origin could have two goals, prevention and treatment. The prevention of obesity of infectious origin could be achieved by vaccination against individual adipogenic pathogens; whereas the treatment may be more difficult and will depend on the adipogenic mechanism of individual pathogens. Antiviral agents may be of help only if the body continues to harbor the pathogen. Antivirals may be useless if the virus operates in a "hit and run" fashion. In such cases, the offending pathogen will have been cleared from the body long before its resulting impact on weight gain is noticed. Such cases will have to be treated by responding to the metabolic consequences of the infection in a genetically susceptible individual.

Understanding the causes and the mechanisms of obesity of infectious origin will be of immense help in individualizing the management of obesity by permitting cause-specific treatments. Recognizing the role of the above-stated pathogens and identifying more such candidates contributing to human obesity is the first step.

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## ABSTRACTS FROM THE LITERATURE

## Dysfunctional Growth Hormone Variant

In order to expand the known mutational spectrum of the growth hormone (GH) gene, 74 patients with familial short stature were screened for mutations in the pituitary-expressed *GH1* gene. Two novel mutations were identified: a missense mutation Ile179Met substitution and a — 360A→G promoter variant; and two other previously known heterozygous lesions were also detected: a Val110Ile variant polymorphism and a Thr-24Ala neutral polymorphism. The Ile179Met variant exhibited a similar degree of resistance to proteolysis and secretion as the wild type GH. Molecular binding studies suggested that the Ile179Met substitution perturbed the interactions between the GH and the GH receptor affecting signaling transduction. This resulted in 50% reduced extracellular related kinase (ERK) activation as compared with that induced by the wild type GH. The authors concluded that these mutations reduced the ERK pathways activation and may thus play a role in mediating GH action of patients with familial short stature (SS).

Lewis MD, Horan M, Millar DS, et al. A novel dysfunctional growth hormone variant (Ile179 Met) exhibits a decreased ability to activate extracellular signal-regulate kinase pathway. *J Clin Endo Metab* 2004;89:1068-1075

**Editor's Comment:** This paper provides the molecular evidence that accounts for the growth failure of some patients with familial SS. It further expands the knowledge of the growth alterations in the presence of normal GH secretion, defined as peak GH levels higher than 10ng/ml after provocative stimulation. There may be other alterations of the GH secretion and/or signaling pathway that lead to SS without altering circulating GH levels. These can be detected in as many as 25% of children with idiopathic SS if appropriately investigated with sophisticated techniques not readily available to the clinician.

The accompanying editorial succinctly reviewed the known molecular alterations associated with SS.<sup>1</sup> If we look we will continue to find other mechanisms accounting for growth alterations and/or SS. The etiology of some SS patients with short parents has been clearly elucidated by these investigators.

Fima Lifshitz, MD

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## Disproving Another Vaccination Scare

The Danish Civil Registration System, implemented since 1968, enabled Hviid and colleagues to perform a very powerful longitudinal study examining the proposed association between vaccination and incidence of type 1 diabetes mellitus (T1DM). Because each Dane is assigned a unique identification number, the population can be followed longitudinally and individual data on different variables can be independently compiled from multiple registry sources, thereby eliminating selection and recall biases. Using this rationale, Hviid et al. followed through December, 2001 all children born in Denmark January 1, 1990 to December 31, 2000 (n = 739,694), and identified 681 cases of diabetes. Using Poisson regression models, the rate ratios for diabetes among children who had received at least one dose of the different vaccines versus unvaccinated children ranged from 0.91 to 1.14 (95% confidence intervals ranged 0.71-1.57). [hemophilus influenza B 1.02; DPT 0.94; DPTP 1.06; MMR 1.08; and oral polio 0.74 (p>0.05)] The rate ratio for maximal number of vaccinations (13) versus no vaccinations was 1.32 [0.42-4.10]. Likewise, the rate ratios did not increase in the 2-4 years after vaccination, the proposed time of disease clustering. Of the 681 cases of diabetes, 26 had siblings with diabetes. Even in this genetically predisposed subgroup (rate ratio for diabetes

in siblings versus no siblings was 40.1 [26.9-59.6]), the rate ratios for diabetes did not significantly increase with vaccination status.

Hviid A, Stellfeld M, Wohlfahrt J, Melbye M. Childhood vaccination and type 1 diabetes. *New Engl J Med* 2004;350:1398-1404.

**Editor's Comment:** The increasing incidence of T1DM has been associated temporally with the widespread introduction of general childhood immunizations. Further, T1DM has been described as clustering 3-4 years after vaccination. This has led some to conclude that vaccination plays a role in the development of T1DM. Associations are NEVER sufficient to prove causation. Thankfully, these authors performed such a terrific study which clarified that childhood vaccinations did not increase the risk of developing T1DM. For recent reviews of the pathogenesis of T1DM, see references 1-3, and reference 4 for immunologic effects of vaccination.

Adda Grimberg, MD

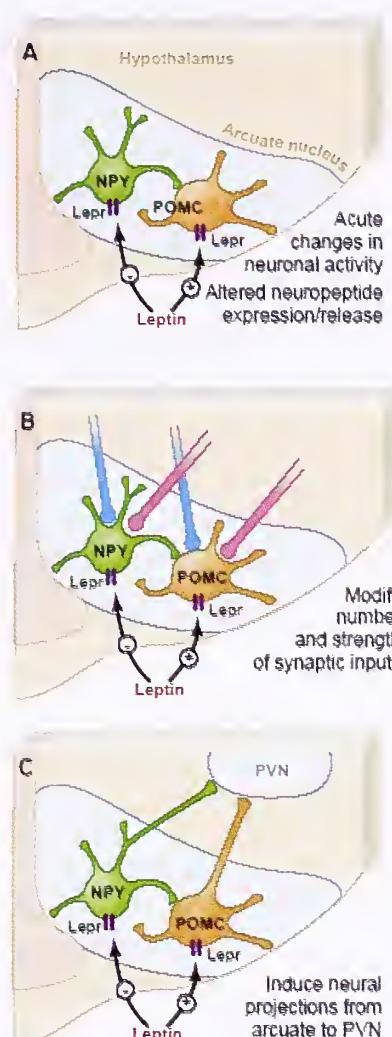
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## Leptin Actions on Hypothalamic Neurons & Arcuate Nucleus

Leptin decreases feeding behavior and encourages weight loss.<sup>1</sup> It stimulates hypothalamic neurons within the arcuate nucleus that synthesize anorexigenic or appetite-suppressing neuropeptides [proopiomelanocortin (POMC) and its products  $\alpha$ -melanocyte stimulating hormone and cocaine- and amphetamine-regulated transcript (CART)]. It also suppresses neurons that synthesize orexigenic or appetite-stimulating neuropeptides [neuropeptide Y (NPY) and agouti-related protein (AgRP)]. These neurons then project to the paraventricular and dorsomedial hypothalamic nuclei, and lateral hypothalamic area (PVH, DMH, LHA, respectively). There, other neuropeptides propagate the feelings of hunger or satiety.<sup>2</sup> Leptin acts directly upon these neurons through the leptin receptor. Pinto et al identified direct anatomical functional effects of leptin upon these arcuate neurons. They transgenetically programmed wild-type (WT) and *ob/ob* mice to co-express fluorescent proteins with POMC (topaz) and NPY (sapphire). As expected each neuropeptide was expressed in a different arcuate neuron. They then examined, by patch clamp recordings in arcuate nuclear slices *in vitro*, the numbers of excitatory and inhibitory afferent inputs into these discrete neurons and quantitated by electron microscopy their anatomically distinct synapses. In *ob/ob* animals, there were far more excitatory than inhibitory impulses into (and excitatory synapses on) NPY neurons than in WT mice. There were many more inhibitory impulses into (and inhibitory synapses on) POMC neurons in *ob/ob* than WT mice. Administration of leptin to *ob/ob* mice reversed these patterns. In WT mice, administration of ghrelin, a gastric appetite-stimulating peptide,<sup>3,4</sup> increased inhibitory and decreased excitatory synapses into/on POMC neurons but did not appear to affect NPY-containing neurons. The authors concluded that there is "neural plasticity" in the arcuate cells containing POMC and NPY and that the effects of leptin and ghrelin are at least partially mediated by such changes.

Bouret et al examined the effect of leptin deprivation and leptin administration upon the density of the neural projections between the arcuate nucleus and the paraventricular nucleus (PVH), dorsomedial hypothalamic nucleus (DMH), and lateral hypothalamic area (LHA) in intact and leptin deficient (*ob/ob*) mice. They placed a "fluorescent ... tracer that labels axonal projections in fixed tissues" into sections of the arcuate hypothalamic nucleus then examined the pattern of fluorescent projections to the target area(s). In WT animals, the density of these projections increased with age. Relative to WT animals at all ages, leptin deficiency was associated with a greatly decreased number of projections from the arcuate nucleus to all target regions, but not to non-target areas. Administration of leptin to *ob/ob* adult mice did not alter this pattern. However, leptin given at very high doses intraperitoneally (1 mg/100 mg body weight IP) between



### Leptin and neurodevelopment.

Leptin acts on the neurocircuitry of the hypothalamus in three ways. (A) Leptin acts directly on the neurons of the arcuate nucleus by binding to the leptin receptors (Lepr) that they express. The altered activity of these neurons in response to leptin results in changes in their production and release of the neuropeptide NPY and the POMC product,  $\alpha$ -melanocyte stimulating hormone. (B) By acting on an unknown site, leptin produces rapid changes in the strength and number of excitatory and inhibitory synapses that have inputs on NPY and POMC arcuate neurons. (C) Leptin induces neurite outgrowth of arcuate neurons, stimulating projections from the arcuate to the paraventricular nucleus (PVN) of the hypothalamus during a critical postnatal period.

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Illustrated by Katharine Sutliff

postpartum days 4 through 12 restored the density of projections to normal by the 80<sup>th</sup> day of life. The authors concluded that leptin is essential for the development of hypothalamic neural pathways that convey leptin downstream signals and that this property was expressed in the neonatal period and perhaps promoted by the neonatal surge in leptin secretion.

Bouret SG, Draper SJ, Simerly RB. Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science* 2004;304:108-110.

Pinto S, Roseberry AG, Liu H, et al. Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science* 2004;304:110-115.

**Editor's Comment:** In an accompanying commentary Elmquist and Flier<sup>4</sup> discussed the significance of the neuroexcitatory and anatomical effects of leptin. They suggested that through the influence of leptin on the excitatory and inhibitory inputs into the arcuate neurons and by stimulation of their neural connectivity, an as yet hypothetical body weight set point might be a functional reality. In mice, there is a surge in leptin secretion in the first week after birth that is not accompanied by a decrease in food intake. The possibility that a body weight set point may be related to and perhaps programmed by the secretion of leptin in the immediate post-partum period (in mice) is intriguing. In human neonates, serum levels of leptin decline over the first 6 days of life and then do not change

appreciably over the first 17 days after birth.<sup>5</sup> Serum leptin concentrations are higher in female than male infants and related to BMI through 12 months of age, but low relative to values in older children and adolescents.<sup>6,7</sup> If there is a set point for body weight as there is for height in man, it is unfortunately easily abridged.

Allen W. Root, MD

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## Attitudes Toward Clinical Management of Intersexuality: The Voices of 46,XY Adult Patients

Controversies regarding the care of individuals born with intersexuality prompted a stream of adult followup studies of psychosocial and psychosexual functioning. Far less attention has been directed at the attitudes held by former patients toward treatment policies. The paper by Meyer-Bahlburg et al represents a marked exception. Specifically, participants were asked about their satisfaction with assigned gender as well as their opinions regarding the desirability of a 'third gender,' and the optimal age for genital surgery.

Attitude data were collected on 46,XY adults who had presented to a pediatric endocrinology clinic with varying degrees of genital ambiguity. The study was a postal survey followed by a physical examination. A total of 72 completed the questionnaire (32 men and 40 women; 18-60 years old). Based upon appearance of the genitalia at time of referral, participants were classified with ambiguous genitalia (AMBI; 21 men, 18 women), micropenis (MICRO; 11 men, 5 women), or female external genitalia (FEG; 17 women). The AMBI group consisted of individuals born with microphallus associated with perineoscrotal hypospadias secondary to various intersex syndromes. MICRO syndromes were attributed to hypergonadotropic hypogonadism, hypogonadotropic hypogonadism, and idiopathic types. The FEG group was made up mostly of patients with complete androgen insensitivity.

Most participants were "mainly satisfied" with assigned gender (85%). In male AMBI and MICRO 68% replied their genitalia appeared unusual, and 76% complained that their penis was too small. Whereas, in female AMBI and MICRO, 39% thought their genitals looked unusual. The majority of participants (73%) were either mainly or somewhat satisfied with sexual functioning.

Only 15% endorsed an assignment of a third gender as a strategy to avoid genital surgery. However, there was a statistical trend for those not satisfied with their own gender to endorse this. When asked about surgical correction of a hypothetical child born with ambiguous genitalia, 67% did not endorse the option of postponing genital surgery until adulthood. When asked to employ hindsight regarding their own genital surgery, 47% thought the procedure should be performed during infancy, 24% recommended postponing surgery until adolescence, and 22% thought

the procedure should have been postponed until their adult years. FEG women almost uniformly endorsed waiting for surgery until adulthood.

Meyer-Bahlburg HFL, Migeon CJ, Berkovitz GD, Gearhart JP, Dolezal C, Wisniewski AB. Attitudes of adult 46,XY intersex persons to clinical management policies. *J Urology* 2004;171:1615-1619.

**Editors' Comment:** Several findings of this study are noteworthy. First, the majority of the 46,XY adult patients with intersexuality expressed satisfaction with assigned gender. This finding has been corroborated in independent studies.<sup>1</sup> Second, 45% were mainly satisfied with their current sexual functioning (while 28% were somewhat satisfied and 27% mainly dissatisfied). Readers should be cautioned against assuming that dissatisfaction with sexual functioning is necessarily related to the quality of the surgical reconstruction. Sexual problems in the general population of men and women are reported to be high.<sup>2</sup> Without a healthy comparison group, the rates of satisfaction/dissatisfaction reported in this study are difficult to evaluate. In addition, the best predictors of sexual distress in women are markers of general emotional well-being and emotional relationship with the partner during sexual activity. In contrast, physical aspects of the sexual response in women, including arousal, vaginal lubrication, and orgasm, are poor predictors.<sup>3</sup> Because survey respondents may assign different interpretations to single questionnaire items, the precise meaning of responses await more detailed assessments. Consistent with patient advocacy groups (eg, the Intersex Society of North America), the majority of survey participants opposed a third gender option. It is reassuring that the message obtained from former patients and patient advocacy groups coalesce in this critical aspect of clinical decision-making.

David E. Sandberg, PhD  
Sherri Berenbaum, PhD

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## Ethics Guidelines for Intersex Conditions

The Hastings Center, explored ethical and social issues raised by surgery aimed at making children appear more typical. A multidisciplinary group considered medical, psychosocial, and ethical issues associated with surgical interventions in children born with atypical genitalia, commonly grouped as intersex.

Parents of newborns with intersex may believe that medical evaluation will reveal their infants "true sex" and that genital surgery should proceed as soon as possible to avoid negative psychological sequelae. However, gender identity is not perfectly predicted by sex chromosomes or other physical parameters. Empirical evidence challenging predictions of positive outcomes from "cosmetic" procedures were reviewed as was diminished sexual responsiveness associated with surgical procedures. The practice of shielding patients from details of their diagnosis and surgical treatment is purported to precipitate disruption of relationships with parents and health care professionals. The authors point out that guidance documents put forth by professional societies are not based on valid clinical investigations and that there exists substantial variability among different specialties. The following conclusions were reached.

- 1) A comprehensive assessment of actual clinical practice should be undertaken.
- 2) Current surgical procedures that normalize genital appearance, are not alone justified. Surgery does not assure that the individual will avoid being discriminated against.
- 3) Appearance-altering surgeries do not need to be performed urgently. Surgical expediency does not necessarily outweigh the psychosocial and ethical considerations of waiting until the patient can participate in decision-making.
- 4) Immediately following diagnosis families require comprehensive services, including access to mental health professionals with intersex expertise. Psychological support is essential.
- 5) To reduce the feelings of humiliation and shame, children should be informed of their differences in an age-appropriate manner.
- 6) Ethical practice demands rigorous follow-up studies focusing on well-being and quality of life. Retrospective studies should include those who have not had surgery and prospective studies should compare the outcomes of non-surgical alternatives. Careful study design is crucial.
- 7) Clinicians need more intersex education including diagnosis, the development of gender, and sexual health.

In an accompanying commentary, Dr. Erica Eugster juxtaposes panel recommendations against the "real life challenges of providing compassionate and responsible care to infants with intersex conditions and their families." Eugster draws attention to the potential psychological risks of postponing genital surgery until the patient is mature enough to provide informed consent. She ponders the implications for parenting of denying the option of early surgery when the family demands surgery. She concludes the ultimate decision should rest with the parents. To safeguard

physical well-being of the child, Eugster reinforces a recent recommendation that genital surgery be undertaken in centers of excellence with intersex expertise.

Eugster welcomes the development of multidisciplinary teams, but recognizes its rare application. Her experience demonstrates that some families passively and/or actively reject counseling. The full integration of counseling services with a multidisciplinary team may temper such resistance. Regarding disclosure of medical information to the child, Eugster welcomes the guidelines for psychoeducational counseling but acknowledges that conflicts may be encountered when family members wish to shield the child from diagnostic details. Eugster enthusiastically endorses increased human sexuality education for clinicians but recommends a targeted strategy of focused training workshops that would serve the purpose of filling staffing gaps in multidisciplinary teams with intersex expertise.

Frader J, Alderson P, Asch A, et al. Health care professionals and intersex conditions. *Arch Pediatr Adolesc Med* 2004;158:426-428.

Eugster EA. Reality vs recommendations in the care of infants with intersex conditions. *Arch Pediatr Adolesc Med* 2004;158:428-429.

**First Editor's Comment:** These papers provide an excellent discussion on the ethical care of patients with intersex conditions. Both pieces underscore the value of outcome studies in guiding clinical practice, and yet the vision of multicenter, multidisciplinary research is largely unrealized. There are inherent limitations on research (eg randomized clinical trial is not an option in assessing the benefits of early versus later genital surgery). Studies examining the relative benefits of multidisciplinary teams versus the current standard of care would be compelling and feasible. The systemic constraints in 'real life' medicine represents even greater challenges. The intense effort required in creating and maintaining multidisciplinary teams serves as a disincentive. Creative problem solving is needed; we will not obtain answers to the most important questions if we restrict our inquiry to those issues that are the easiest to study.

David E. Sandberg, PhD

**Second Editor's Comment:** The reader is encouraged to review the article "Discordant sexual identity in some genetic males with cloacal exstrophy assigned to female sex at birth",<sup>1</sup> as well as the accompanying commentaries by many experts regarding sex determination, differentiation, and identity.<sup>2</sup> Altogether these articles should be carefully considered when caring for these patients. As Eugster stated, "The most important determinant of outcome may be an individual family's ability to accept and unconditionally love their child."<sup>3</sup>

Fima Lifshitz, MD

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## IGF-1 and Cancer Risk

The authors undertook a meta-analysis of 26 published (and closely scrutinized) data-sets examining the relationship between circulating concentrations of IGF-I and IGFBP-3 and the risk of developing prostatic, breast, colon, and lung cancer in adult men and women employing extremely strict, unified, and sensitive analytical methods. After stratification of the analyte levels, they compared the 75<sup>th</sup> percentile of circulating protein concentration with the 25<sup>th</sup> percentile and then calculated odds ratios for the development of cancer. There were significant associations between the concentration of IGF-I and development of premenopausal breast, prostatic, and colon cancer (odds ratios: 1.93, 1.83, and 1.58, respectively). There was no association between IGF-I values and risk of breast cancer in postmenopausal women or of lung cancer. The IGFBP-3 level was associated with an increased risk of development of breast cancer in premenopausal women (odds ratio 1.96) and possibly with a protective effect on development of lung cancer. The investigators concluded that because of the proliferative and anti-apoptotic effects of various IGFs, higher circulating concentrations of IGF-I are a risk factor for development of 3 non-smoking related common malignancies.

Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. *Lancet* 2004; 363:1346-1353.

**First Editor's Comment:** The association of hypersomatotropism with increased risk for development of cancer of the colon is well known.<sup>1</sup> The present report concluded that higher IGF-I concentrations contributed to the development of several malignancies. The mechanism(s) by which IGF-I enhanced or facilitated neoplasia and the roles that IGFBPs and IGFBP proteases (such as prostate specific antigen) played in this process are not known with certainty.<sup>2</sup> The precise point at which a malignancy began and the corresponding IGF-I value is unknown. However, the association between IGF-I concentrations and malignancy should give one pause when recommending the use of growth hormone (GH) in short subjects without GH deficiency, particularly in the absence of data indicating that an increment in adult height of 2 or more inches results in greater academic, social, and economic well-being.

Allen W. Root, M.D.

**Second Editor's Comment:** The major lesson from the IGF/cancer association is to beware of over generalizations; this is a story of complicated nuances. Over 200,000 patient-years' experience globally with recombinant human growth hormone (rhGH) therapy revealed a very strong safety profile. This safety profile may not hold as our use moves from purely physiologic replacement to increasingly pharmacologic use, in terms of both higher doses and newer indications. For example, increased mortality ensued when rhGH was administered to critically ill adults in an attempt to foster anabolism.<sup>3</sup> Conversely, we cannot jump to the conclusion that IGF-1 (and GH) are necessarily harmful. While high IGF-1 levels have been associated with higher cancer risk, low IGF-1 levels have been associated with increased risk for age-related memory loss, Alzheimer's dementia and diabetes-associated dementia.

The underlying science is also filled with nuances that likely contribute to the contradictory results found in the literature. The relative contributions of endocrine (circulating) versus autocrine or paracrine GH/IGF axis components to cancer progression remain unclear and technical issues, such as IGFBP interference with some IGF assays<sup>4</sup> and interassay variations,<sup>5</sup> can cloud the results. Due to its dynamic nature, measuring components of the IGF system may yield different results, ie a shift in the amount of free versus total IGF, the IGFBP profile, or the amount of intact versus proteolyzed IGFBP.

More research is needed, as in long-term surveillance of the rhGH recipients into late adulthood when the natural incidence of cancer increases. As a safety marker, IGF-1 levels should be closely monitored in all rhGH recipients to avoid supraphysiologic concentrations. Clinical nuances may also be explored regarding potential risk modulation by altering rhGH dose or duration of treatment.<sup>4</sup> Ultimately, treatment should be individualized and tailored to the risk/benefit analysis for each patient. That includes appreciating the true benefit (or not) of height augmentation.

Adda Grimberg, MD

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## Novel Treatment for Osteogenesis Imperfecta

Osteogenesis imperfecta (OI) is a relatively common genetic disorder characterized by bone fragility, skeletal deformities, ligamentous laxity, thin skin, blue sclerae, and other "connective tissue" features. It results from heterozygous mutations of the *COL1A1* and *COL1A2* genes that encode the pro $\alpha$ 1 and pro $\alpha$ 2 chains of type I collagen; its manifestations reflect the distribution of type I collagen. The most severe forms of OI, eg, OI type II, result from mutations in which the mutant procollagen chains are synthesized, participate in and disrupt the assembly of triple helical type I collagen molecules. Such mutant genes or alleles might be considered dysfunctional alleles. In contrast, milder forms, such as OI type I, result from mutations that inactivate one of the two *COL1A1* or *COL1A2* alleles, leaving the patient with only one functional *COL1A1* or *COL1A2* allele. Accordingly, one strategy to treat severe OI is to convert the dysfunctional *COL1* allele to a nonfunctional or null allele. A group headed by David Russell (Chamberlain et al) has demonstrated the feasibility of inactivating dysfunctional *COL1A1* alleles in adult, or often termed mesenchymal, stem cells (MSC) from patients with severe OI.

MSC available from the marrow of bone samples discarded at surgery, have the potential to repopulate bone marrow and locally generate new bone. In principle, MSC could be harvested from a patient with severe OI, genetically manipulated to inactivate a dysfunctional type I collagen gene, and be transplanted back into the patient where they would home to bone marrow and locally produce bone tissue with properties of mild OI.

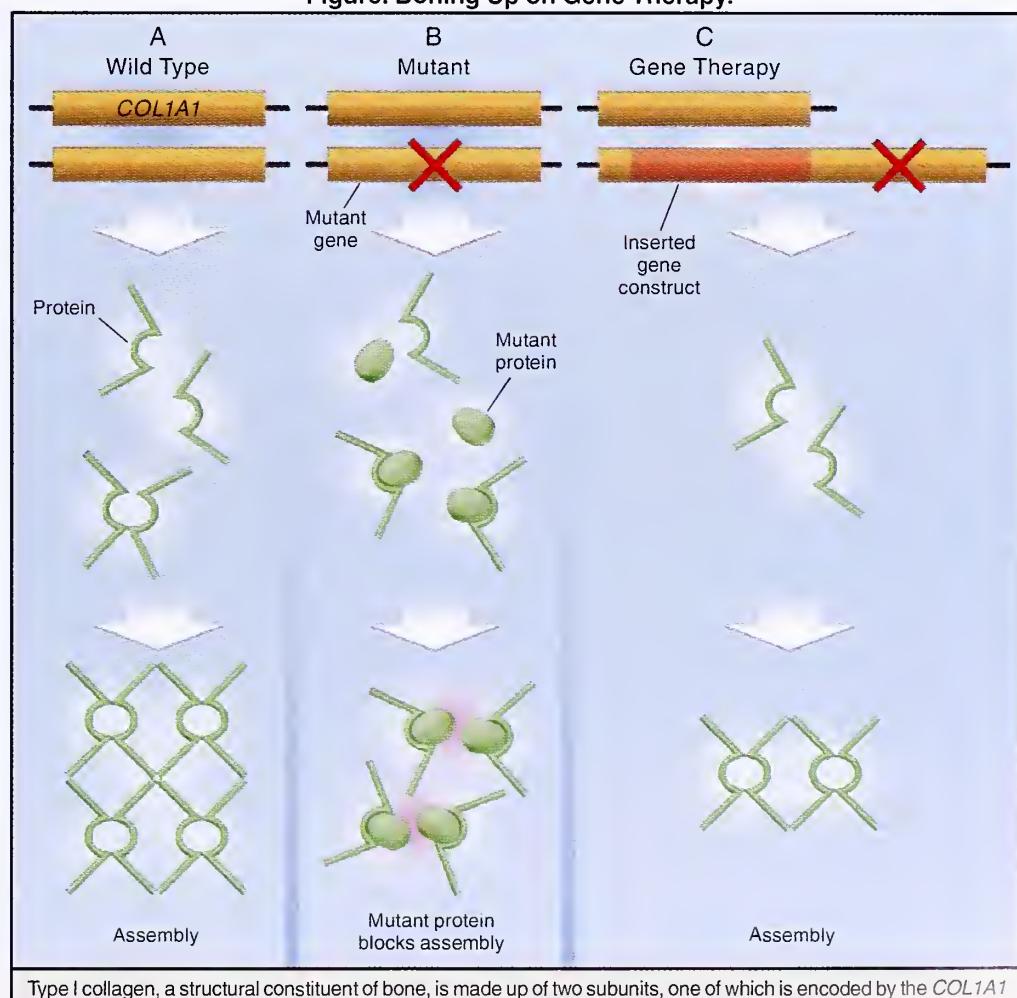
In this investigation, MSC were isolated from 2 patients with severe OI whose specific mutations were identified and shown to be of the dysfunctional type. The cells were infected with an adenovirus-based gene targeting vector designed to promote homologous recombination between the vector and the *COL1A1* gene. Successful targeting inserted foreign DNA, including the gene for neomycin resistance, into exon 1 of the *COL1A1* gene, thereby preventing its expression and converting it to a null allele. The targeting vector did not distinguish between the mutant and normal *COL1A1* allele. The MSC were then grown in an antibiotic to select recombinant cells and then analyzed to confirm that targeting was successful. Analysis of several cell clones, as well as pools of cells, revealed that a high percentage of the resistant MSC had undergone targeting at one *COL1A1* allele. Significant improvements

were observed in measures of collagen processing, stability, and fibril ultrastructure for targeted cells, and accumulation of suspected mutant collagen present in the original cells disappeared in the targeted MSC. To determine if the targeted MSC retained their ability to become osteoblasts, the targeted MSC were implanted subcutaneously into immunodeficient mice, removed after 8 weeks, and analyzed. Although amount varied, bone formation was detected in all of the implants and the osteocytes were shown to be of human origin.

The authors note potential problems with their approach, such as inability to specifically target the mutant versus the normal *COL1A1* allele and possible immunogenicity of the foreign neomycin-resistance gene. But they argue that these can eventually be overcome and that their approach has certain advantages over allogeneic bone marrow and MSC transplantation, which has been reported in a clinical trial for severe OI.

Chamberlain JR, Schwarze U, Wang P-R, et al. Gene targeting in stem cells from individuals with osteogenesis imperfecta. *Science*. 2004;303:1198-1201.

Figure. Boning Up on Gene Therapy.



Type I collagen, a structural constituent of bone, is made up of two subunits, one of which is encoded by the *COL1A1* gene (Panel A). Mutations in the genes encoding these subunits cause osteogenesis imperfecta. Dominant negative mutations result in mutant protein that interferes with the activity of wild-type protein encoded by the unaffected gene (Panel B). Diminishing the levels of mutant protein may be a way to treat the disease, and a recent study by Chamberlain et al provides support for this approach and suggests that it could be achieved through gene therapy. Using a molecular construct engineered to insert itself into the *COL1A1* gene (Panel C), the investigators showed that the processing and stability of collagen could be improved by the targeting of mutant mesenchymal stem cells, and that the targeted cells become bone cells. Although the construct probably targets mutated and wild-type alleles equally, the approach works because the cells in which the wild-type allele is inactivated do not produce collagen, and the cells in which the mutated allele is targeted produce adequate amounts of the normal protein.

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**First Editor's Comment:** There are 2 major challenges in order for this form of genetic treatment to be successful. The first is being able to alter the mutant gene so that the mutation is corrected or nullified, as in this case. This paper demonstrates that conversion of a dominant-negatively acting allele to a null allele works, at least in cell culture and in mice, and can be carried out in a time frame that is realistic for clinical use. Although there are still many problems to resolve, the gene-targeting strategy has considerable promise. The second challenge is to achieve sufficient engraftment of genetically modified cells to repair excessively fragile bones. Fortunately, therapists can exploit the high vascularity of bone and the natural behavior of MSC to home to marrow and differentiate as functional osteoblasts. However, previous attempts at allogeneic MSC transplantation and similar experiments in mice have resulted in modest engraftment, at best. Figuring out how to safely and effectively impart therapeutic cells with a competitive advantage over their

dysfunctional endogenous counterparts in bone may prove to be the greater of the 2 challenges. Nevertheless, given the absence of other successful treatments for severe OI, it remains a viable potential option.

William A. Horton, MD

**Second Editor's Comment:** The exciting field of gene therapy has been given a shot in the arm by these studies. The recent comment by Prockop<sup>1</sup> on targeting gene therapy for OI is worth reading in order to facilitate the appreciation of this novel concept (figure).

Fima Lifshitz, MD

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## Teasing Apart GH from IGF-I Effects on Longitudinal Bone Growth

Wang and colleagues examined tibial growth in mice with targeted deletions of the insulin-like growth factor-I gene (*Igf1*) or growth hormone (GH) receptor gene (*Ghr*) to elucidate the direct versus indirect (ie IGF-I-mediated) effects of GH on longitudinal bone growth. The study design was based on the fact that *Igf1*<sup>-/-</sup> mice do not produce IGF-I in either the circulation or local tissues, but have high levels of GH due to the loss of IGF-I negative feedback. They would therefore be expected to retain any IGF-I-independent effects of GH action. In contrast, *Ghr*<sup>-/-</sup> mice lose all GH effects. The authors focused on tibial growth from postnatal days 20 to 40, a period of maximal GH action in normal murine growth which precedes sexual maturity. Further, because the two genetic mutants were created in different background mouse strains, all results were analyzed as a percent of the wild-type littermates. This controls for both genetic variations between the two strains and for any uterine or environmental factors that may affect growth.

Body weights of both mutants were about 60% less than wild-type littermates. Tibial morphology remained grossly normal in both, but the tibial growth rate was about 37% less in *Igf1*<sup>-/-</sup> mice and 65% less in *Ghr*<sup>-/-</sup> mice. The germinal zone, the upper growth plate region that produces chondrocyte precursors, was enlarged in *Igf1*<sup>-/-</sup> mice but smaller in *Ghr*<sup>-/-</sup> mice, suggesting IGF-I-independent effect of GH. IGF-II mRNA levels, as assessed by *in situ* hybridization, were increased in the former and decreased in the latter mutants. Similarly, the proliferative zone was unaffected in *Igf1*<sup>-/-</sup> mice but diminished in *Ghr*<sup>-/-</sup> mice; here, too, IGF-II mRNA was increased in the former but decreased in the latter. In contrast, the hypertrophic zone was markedly

reduced in both mutants. It remains unresolved whether prechondrocyte proliferation is directly enhanced by GH or by GH-induced local IGF-II production.

Wang J, Zhou J, Cheng CM, Kopchick JJ, Bondy CA. Evidence supporting dual, IGF-I-independent and IGF-I-dependent, roles for GH in promoting longitudinal bone growth. *J Endocrinol* 2004;180:247-255.

**Editor's Comment:** Confirming similar results in femoral studies of different genetic mouse strains, this paper nicely demonstrated IGF-I-independent effects of GH on chondrocyte production and proliferation, and IGF-I-dependent effects on chondrocyte hypertrophy in murine tibial growth plates. It also opens the possibility that the IGF-I-independent effects may be mediated by GH-induced local production of IGF-II. Similar analyses in *Igf-II*<sup>-/-</sup> mice will be needed to answer this question, as are additional experimental models to determine the contribution of IGF-II in a physiologic context as opposed to a possibly compensatory role when IGF-I is deleted. A study comparing *Igf1*<sup>-/-</sup>, *Igf-II*<sup>-/-</sup> and GH deficient *lit/lit* mice found a greater contribution of IGF-I than IGF-II to bone mineral accretion and pubertal bone growth.<sup>1</sup> Thus, more than 10 years after the first description of the *Igf1*<sup>-/-</sup> and *Igf-II*<sup>-/-</sup> mice continue to teach us.<sup>2</sup> For an excellent review of the GH/IGF system in controlling somatic growth, see reference 3.

Adda Grimberg, MD

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## Transient Adrenocortical Insufficiency of Prematurity

There have been several reports of very low birthweight (VLBW) infants who experience systemic hypotension that is unresponsive to volume expansion and inotropic agents, but very responsive to corticosteroids. Ng et al have performed a prospective study of the pituitary adrenal axis in 137 VLBW infants, of whom 78 had refractive hypotension (group 2) and 59 remained normotensive (group 1). Human corticotropin releasing hormone (hCRH) (1mcg/kg IV bolus) was administered between 08:00 h and 10:00 h on days 7 and 14 of life. Serial samples for ACTH and cortisol were obtained at baseline, 15, 30, and 60 minutes after injection. Inclusion criteria were gestational age <32 weeks, birthweight <1500 grams, no postnatal systemic or inhaled corticosteroids, and an indwelling arterial line. Exclusion criteria were persistent hypoglycemia, systemic infection, necrotizing enterocolitis, or major surgery.

Results from groups 1 and 2 combined, showed that basal and peak cortisol and change in cortisol over the first 30 minutes after hCRH injection correlated significantly with the lowest recorded BP during the first 14 days of life and the BP measured at the initiation of the study on day 7. In contrast, ACTH levels on days 7 and 14 and cortisol on day 14 did not correlate with the lowest BP. Serum cortisol levels (after hCRH) on day 7 correlated negatively with the total dose of inotropic agents, while plasma ACTH levels were positively correlated.

The ACTH response to hCRH was significantly greater on both days 7 and 14 in group 2 infants, but cortisol responses were greater in group 1 than group 2 on day 7. Day 14 cortisol responses were similar in both groups. The authors state that this study demonstrates adrenal hyporesponsiveness in group 2 infants at day 7. Those

were the infants with significant hypotension requiring inotropic agents. By day 14, the transient nature of this endocrine dysfunction was evident as there were no significant differences between the two groups of infants. The authors term this dysfunction, transient adrenocortical insufficiency of prematurity or TAP.

Ng PC, Lee CH, Lam CWK, et al. Transient adrenocortical insufficiency of prematurity and systemic hypotension in very low birthweight infants. *Arch Dis Child Fetal Neonatal Ed* 2004;89:F119-F126.

**Editor's Comment:** This is an interesting, well-conducted prospective study of a problem that is relatively common in many NICUs. Neonatologists have been using small doses of hydrocortisone in premature babies with hypotension refractory to inotropic agents for some time. However, the definition of the defect responding to this non-replacement, non-stress level of hydrocortisone administration has not been clarified. Ng et al have provided a clear demonstration that these infants have a transient adrenal, not pituitary immaturity, which requires hydrocortisone administration. They note that a previously reported trial of hydrocortisone versus dopamine for the routine treatment of hypotension failed to confirm its benefit. This is not surprising, given the distinct differences in the 2 groups of infants studied. A short course of hydrocortisone in premature infants with hypotension refractory to inotropic agents seems a reasonable therapeutic maneuver. Data now show that these corticosteroids do not need to be given for prolonged periods.

William L. Clarke, MD

## Long-term Mortality in the U.S. of Pituitary-derived Growth Hormone Recipients

Mills and colleagues from the NIH, FDA, and CDC presented long-term mortality data on patients who received pituitary-derived growth hormone (pGH) from the National Hormone and Pituitary Program (NHPP) during the years 1963–1985. Data through December 1996 were obtained for 6107 of the 6272 children who received pGH. Information regarding the reason for pGH treatment and the specific cause of death was obtained. Death certificates were reviewed in all but 3 instances. Causes of GH deficiency were categorized as idiopathic, organic (including tumor-related or non-tumor related—eg septo-optic dysplasia, histiocytosis, trauma, etc.), or other (including unknown causes, neurosecretory defect, Turner syndrome, etc.). Subjects were classified as having isolated GH deficiency, multiple hormone deficiencies, unspecified deficiency (insufficient information to classify), or not applicable (non-GH deficient). Subjects with adrenal insufficiency and/or a history of hypoglycemia were identified.

Observed mortality was compared to that expected in a similar US cohort. Relative risks were calculated and a proportional hazards model constructed.

There were 433 deaths from 1963–1996 compared to an expected number of 114. Thus the overall risk of death was nearly 4 times that of the general population (RR, 3.8; 95% CI, 3.4–4.2;  $p < .0001$ ). Only subjects with idiopathic isolated GH deficiency had a death rate similar to that expected for the population at large. The highest risk categories included patients with either benign or malignant tumors, adrenal insufficiency, and hypoglycemia. Tumors, hypoglycemia, adrenal insufficiency, and multiple hormone deficiencies were demonstrated to be significant, independent risk factors by proportional hazards analysis. There were 26 deaths from Creutzfeldt-Jakob disease (CJD). Two deaths were from colorectal cancer; one of whom had familial polyposis and the other had received radiation for a CNS tumor. One subject died from Hodgkin's disease. Thus, the reported associations between GH therapy and colorectal

cancer or Hodgkin's disease were not observed.

Of interest is that 24.5% of the deaths were sudden and unexpected. Of those, multiple hormone deficiencies were present in at least 74%, a history of hypoglycemia was present in 31% and seizures had occurred in 52%. Deaths followed a clinical course suggestive of adrenal insufficiency in 56% of deaths. Sudden unexpected death was also associated with the presence of a medical problem other than isolated GH deficiency—craniopharyngioma (24%) or other intracranial tumors (14%). Hypoglycemia in children was associated with a 9 fold increase in risk. The death rate in those with adrenal insufficiency remained stable as children aged.

The authors emphasized 3 findings. First, hypoglycemia was an important risk factor for death, which decreased as the children aged and presumably could identify and treat their own symptoms. Second, tumors were an important cause of death, even though the risk of colorectal cancer, Hodgkin's disease and overall cancer deaths were not increased. Third, adrenal insufficiency was an unexpected high-risk factor leading to death even in adulthood. They stated that increased steroid doses for even supposedly trivial infections was important as 30 of these 35 subjects were found dead or comatose and most likely died of unrecognized or inadequately treated adrenal insufficiency.

Mills JL, Schonberger LB, Wysowski DK, et al. Long-term mortality in the United States cohort of pituitary-derived growth hormone recipients. *J Pediatr* 2004;144:430-436.

**First Editor's Comment:** This report presents some alarming and some re-assuring information. That patients who had received pGH have a markedly increased relative risk of dying is alarming. That the cause of their deaths, in many instances could be prevented by appropriate glucocorticoid administration for rather trivial infections suggests that endocrinologists are not teaching

or reminding patients of the importance of increasing their medications or seeking medical assistance at the first sign of infection. The reassuring news is that there does not appear to be an increased risk of colorectal cancer, Hodgkin's disease or other cancers in this cohort. Furthermore, there have been no new cases of CJD in subjects who began pGH treatment after 1977.

These data are interesting and compelling and deserve to be read by those who care for these children. At quick glance, it might appear that pGH administration was a dangerous treatment. On closer inspection, the facts are much friendlier. One can anticipate discussing these findings with parents of these patients.

William L. Clarke, MD

**Second Editor's Comment:** This is an important paper which clearly documents higher mortality risks of hypopituitary patients and the surprisingly high number of unexpected sudden deaths. The concern with CJD is justified and requires continuous surveillance, but there isn't much we can do to prevent it in those who harbor the prion. However there is a lot we must do when treating hypopituitary patients to prevent unexpected fatalities. Adrenal insufficiency must be aggressively treated particularly in patients who vomit when ill. It is not clear why the patients in this report failed to do so, but it is clear that more emphasis is needed so patients receive appropriate steroid replacement during periods of stress. Familiarity breeds complacency—or so it seems. However, there were 20 sudden deaths in patients without adrenal insufficiency. The possibility that uncontrolled diabetes insipidus played a role should also be kept in mind, particularly when oral DDAVP therapy may not be effective, such as when a patient vomits.

Fima Lifshitz, MD

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## PREGNANCY IN ADOLESCENTS WITH TYPE 1 DIABETES

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### INTRODUCTION

Adolescence is a time of many changes. For an adolescent with type 1 diabetes mellitus (T1DM), change means becoming more self-reliant in dealing with chronic illness. Rebellion, acting out, and the desire to be "normal" may drive the adolescent with chronic disease to make poor choices.<sup>1,2</sup> Such choices may have detrimental effects on dietary intake, medication usage, and social behavior.<sup>1,3</sup> For diabetic adolescents, low self-esteem and depression may contribute to behavior resulting in an unplanned pregnancy. This is of high risk to both the woman and fetus and is associated with high rates of congenital malformations, spontaneous abortions, and stillbirths.<sup>4,5</sup> Additionally,

### From The Editor's Desk

Volume 20, number 4, of *GGH* marks the first year of the journal under my direction. I am proud of what we accomplished and I thank the editorial board for their support. The scope of pediatric endocrinology keeps on diversifying and with it, the journal's contents. The lead articles of the 2004 volume reviewed important subjects, some beyond the immediate concerns of our colleagues. In this issue the topic of pregnancy in adolescents with type 1 diabetes mellitus by Brindley & Jovanovic addresses the risks and consequences of adolescent pregnancy; these may be costly to both the mother and the fetus, thus underlining the importance of dealing with contraception as part of the treatment of our patients. Also included are the abstracts and editorial comments of exciting papers selected by our editorial board, these deal with pertinent clinical concerns and basic discoveries in the etiopathology of patients encountered in pediatric endocrine practice. Please note the **new feature** introduced to the journal in this issue, namely the electronic abstracts which are only displayed on the website. This new feature allowed the publication of important abstracts with erudite comments; these could not be included in the printed version of the journal because of space limitations. These e-abstracts are concurrently listed in the table of highlights with those published in both the printed and the electronic version of the journal.

During the last year, we accomplished a tremendous growth in the number of subscribers that enjoy *GGH* through the Web and we welcome over 1700 new readers. The reach of the journal also increased, over 37% of our online readers are now from countries beyond the United States, almost a 45% increase in worldwide distribution, with an excess of 30,000 visitors to date. However, we were often challenged with wrong email addresses and returned notifications. The protective filters pose obstacles to the exchange of legitimate scientific information through the internet. We no longer include a Table of Contents in the email announcement as this may trigger filters (ie, intersex). Thus, I want to remind our subscribers to please inform us of email address changes and to notify their I.T. staff to allow [www.GGHjournal.com](http://www.GGHjournal.com) through the institutional filter systems.

I am pleased to inform you that we **will not** discontinue the printed version of the journal as was planned. It will be published and distributed by surface mail within the United States. Finally, a word of thanks to our sponsor, Genentech Inc., for their continuous support through an unrestricted educational grant award for the publication of *GGH*.

Respectfully,  
Fima Lifshitz, MD

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### E-Abstracts (Abstracts On Line)

Bardet-Biedl Syndrome – Cellular Disorganization
Filamin B Mutations Disrupt Skeletogenesis in Bone Dysplasias
Raloxifene and Tamoxifen Treatment of Pubertal Gynecomastia

the pregnancy can complicate diabetes. Retinopathy and nephropathy may worsen,<sup>6,7</sup> and preeclampsia and hypertension of pregnancy occur more frequently.<sup>8</sup> However routine physician visits usually focus on the state of the disease without addressing the sexual habits and/or contraceptive options for adolescents. Planned pregnancies are not relevant for most teenagers, thus pregnancy is usually unintended.<sup>9,10</sup> Medical intervention usually begins after embryogenesis and organogenesis,<sup>11</sup> and the level of glycemic control, is often sub optimal at the time of conception and early development.<sup>9,12</sup> This review aims to bring to the attention of pediatric endocrinologists the importance of this issue.

## MENARCHE AND MENSTRUAL DISTURBANCES

The hypothalamic-pituitary-ovarian axis is often incompletely mature in adolescents with T1DM<sup>13</sup> resulting in delay of menarche, irregular menses, and secondary hypogonadotropic amenorrhea, oligomenorrhea or polymenorrhea. Those with poorer control were those with oligomenorrhea/amenorrhea. Strotmeyer, et al<sup>14</sup> also reported a highly significant difference between age of menarche in patients with debut of diabetes before age 10 years compared to healthy controls and sisters (Table 1).

Moreover, poor metabolic control of T1DM is associated with worsening menstrual disturbances.<sup>15</sup> Diabetic adolescents with irregular menses, primary amenorrhea, secondary amenorrhea, or oligomenorrhea had a significantly higher glycosylated hemoglobin (A1C) level (11.4% vs 9.7%), than diabetic adolescents with regular menses.<sup>15</sup> As the A1C value increased above 10%, the prevalence of menstrual disturbances also increased; when the glycemic control improved, menstrual regulation ensued.<sup>16</sup> Diabetic adolescents with irregular cycles had a mean A1C of 12.8%, compared to a mean of 10.5% in those with regular cycles. Poor glycemic control is unfortunately a common problem

in adolescents with T1DM.<sup>3,17,18</sup> The mean A1C in children was 8.6%<sup>17</sup> and in adolescents peaked at 9% to 9.5%. In the United Kingdom, the mean A1C was 9.1%, with less than 15% of pediatric and adolescent patients having an A1C level <8.0%.<sup>18</sup> Poor glycemic control among adolescent diabetic patients is also associated with other issues that compound the control of the disease.<sup>1,3,19</sup> The prevalence of eating disorders, anorexia nervosa and bulimia nervosa in adolescent females with T1DM is increased compared to age-matched controls. Those patients with an eating disorder (DSM-IV criteria) had a higher A1C level than those who did not (9.4 vs 8.6%).<sup>1,19</sup> Additionally, psychiatric disorders such as anxiety and depression are more common in female adolescents with chronic disease than in their healthy counterparts.<sup>20</sup> Although not specific to diabetes, the Adolescent Health Survey of Barcelona,<sup>20</sup> reported significantly elevated rates of low self esteem, personal problems, and feeling sad in chronically ill adolescents. A similar increase in psychological disorders occurred more often in adolescents with T1DM and was associated with poorer glycemic control.<sup>21,22</sup>

The changing insulin needs of adolescents as they mature through puberty may also contribute to the tendency for poor glycemic control. The peak insulin requirement (up to 2 units/kg/day) occurs at Tanner Stage 3. As the diabetic young woman progresses through Tanner Stage 5, there is a gradual reduction in insulin requirements. Insulin misuse or insulin omission may also be used as a weight-control method.<sup>1,3,19</sup> As a result, glycosuria increases and the sense of being able to eat "anything" may be reinforced. On the other hand, when the daily insulin dose is high, there is a tendency for weight gain.

## UNPLANNED PREGNANCY

Adolescents with T1DM are as likely as non-diabetic adolescents to engage in unprotected sexual activity. However, at their medical appointments, physician visits are more likely to focus on the state of T1DM, compliance with medical regimens, and laboratory data, and not deal with birth control and/or contraceptive usage. Chronically ill adolescents are less likely to receive contraceptive counseling and sexual education than healthy counterparts.<sup>23,24</sup> Young women with T1DM are less likely to receive the most effective hormonal contraceptive, a combined estrogen-progesterone pill, than those without the disease.<sup>24-26</sup> Other often less effective methods of contraception such as condoms, IUDs, and surgical sterilization are more often recommended for diabetic women than for non-diabetic women.

Furthermore, T1DM adolescents may, in an attempt at independence, act in ways that are counterproductive.<sup>1</sup> This may include changing medications or dosing schedules, eating forbidden foods, experimenting with drugs or alcohol or engaging in other behaviors that are risky to their health. For some adolescents with diabetes,

**Table 1**  
Descriptive characteristics of women with and without T1DM<sup>14</sup>

	T1DM	Without Diabetes Sisters	Controls	p
n	143	186	158	
Age at menarche (years)	13.5 ± 1.9	12.5 ± 1.4	12.6 ± 1.4	<0.001
Ever oral contraceptive use (%)	44.0	79.0	79.8	<0.001
Mean number of pregnancies*	2.3 ± 1.6	2.9 ± 1.4	2.6 ± 1.4	<0.001
Miscarriages (%)*	31.2	32.1	27.9	0.76
Stillbirths (%)*	10.1	0.6	0.9	<0.001
Ever smoked (%)	41.8	48.4	50.0	0.33
College attendance (%)	64.6	65.6	75.9	0.06
Income >\$40,000 (%)	40.8	59.1	52.7	0.006
Mean BMI (kg/m <sup>2</sup> )	24.6 ± 4.5	25.2 ± 5.3	27.4 ± 7.3	0.003

Data are mean ± SD.

\* excluding women who had never been pregnant

Adapted from Strotmeyer ES, Steenkiste AR, Foley TP Jr, Berga SL, Dorman JS. *Diabetes Care* 2003; 26:1016-1021.

pregnancy may be the only way to prove that one is a "normal" adolescent female.

The United States and the United Kingdom have the highest rates of teenage pregnancy in the world; in the U.S., 52 of 1000 adolescents between the age of 15 and 19 gave birth in the year 2000.<sup>27</sup> Within the first month of initial intercourse, 20% of adolescent young women become pregnant and nearly 50% have a second pregnancy while in their teenage years.<sup>9</sup> Young women with diabetes are more likely to become pregnant than their age-matched controls (ages 16–24), or age-matched young women with phenylketonuria, another chronic metabolic disorder with strict dietary control issues. They are more likely to have been pregnant before, but not more likely to have given birth.<sup>10</sup>

Adolescent pregnancy is associated with higher than expected rates of intrauterine growth retardation (IUGR) and premature births.<sup>28,29</sup> Low birth weight, preterm delivery, small for gestational age, and other malformations were associated with maternal age <18 years.<sup>29</sup> Poorly controlled diabetic women have higher rates of perinatal mortality and fetal malformations than nondiabetic women.<sup>5,30</sup> Although the data on pregnant women with diabetes is obtained from groups involving various age groups, the adolescent with T1DM may be at a greater risk.<sup>31</sup> Lack of dietary folate supplementation prior to conception, as well as lack of proper nutrition, inadequate weight gain, and poor metabolic control may contribute to poor pregnancy outcome in T1DM.<sup>31,32</sup> Inadequate weight gain during pregnancy also increases risks of neural tube defects.<sup>33,34</sup> Other negative factors include lack of pregnancy planning and delayed access to prenatal care or poorly attended prenatal classes; the majority of such pregnancies occur in unwed and poorly educated young women.<sup>28,29</sup>

### EFFECT OF DIABETES ON PREGNANCY

Unplanned pregnancies are often complicated in healthy teenagers, but pregnant adolescents with chronic diseases are at greater risk. Pregnancy in T1DM is considered a high risk to both the woman and the fetus. In these pregnancies, the rates of pregnancy-induced hypertension, preeclampsia, premature delivery, and cesarean section were more than 4-fold the rates observed in the non-diabetic population. Also, the prevalence of infants born large for gestational age was much higher (20% vs 3.5%) and the gestational age was significantly less. Elevated maternal A1C level early in pregnancy was an independent risk factor for pregnancy-induced hypertension and preeclampsia.<sup>8</sup> Moreover, the presence of diabetic nephropathy (defined as persistent proteinuria or albuminuria >300 mg/day) in the first 20 weeks of pregnancy, was associated with an increased risk of IUGR, fetal distress, and preeclampsia. Preterm deliveries and/or cesarean section births were increased as well. The presence of microalbuminuria (30–300

mg/day) can also complicate the pregnancy of T1DM patients; they present increased rates of preeclampsia, preterm births, and infants with IUGR.<sup>35</sup> The pregnancy of a diabetic young woman may also be complicated by the use of multiple medications. Treatment of chronic hypertension or pregnancy-induced hypertension may be required.<sup>36</sup> Unfortunately compliance with medical regimens is low in T1DM adolescents,<sup>1,3</sup> making the treatment of hypertension challenging.

Maternal hyperglycemia has been shown to complicate pregnancy more than any other factor. A1C levels at the time of fertilization and embryogenesis have been linked to a higher rate of spontaneous abortions and congenital malformations.<sup>5</sup> Those with A1C levels >7.5% had a 4-fold increase in spontaneous abortions and a 9-fold increase in congenital malformations. The risks of diabetic ketoacidosis (DKA) during pregnancy include life threatening metabolic derangements for the woman and intrauterine demise for the fetus.<sup>37</sup> DKA in adolescents usually results from insulin omission and infection.<sup>38</sup> Furthermore, the tightly controlled blood sugars recommended in pregnancy<sup>39,40</sup> increase the risk for maternal hypoglycemia and fetal injury. Of interest is that pregnant T1DM women have a lower risk of miscarriage and of delivering infants with birth defects and congenital malformations than women with T2DM.<sup>41</sup>

### EFFECT OF PREGNANCY ON DIABETES

While diabetes can complicate pregnancy, the pregnancy itself may complicate the woman's diabetes. The ever-changing insulin needs of a pregnant diabetic can be very difficult to meet, even for the most dedicated patient. The diabetic adolescent is challenged to strictly follow the dietary demands of pregnancy and the rigorous insulin regimens. There are also medical complications of diabetes that may develop and/or worsen during pregnancy. Young adults with a mean duration of diabetes of 12.7 years were shown to have retinopathy (70% background and 10% proliferative) at baseline.<sup>42</sup> Bouhanick et al reported a retinopathy rate of 50% 15 years after the onset of disease.<sup>43</sup> The Diabetes in Early Pregnancy Study<sup>44</sup> assessed the progression of retinopathy with fundus photography early after conception and followed through 1 month postpartum. There was progression to retinopathy in 10% of those who had none to start with, and in 50% of those who had baseline moderate-to-severe non-proliferative retinopathy. The Diabetes Control and Complications Trial (DCCT)<sup>6</sup> also reported an increased risk of progression of retinopathy in both the conventionally treated and in the intensively treated group of pregnant women. The proposed mechanism of the progression of this complication is either suboptimal control or a rapid change in the control of the illness that occurs early in pregnancy. Elevated A1C at baseline and degree of improvement of glucose control through week 14 were found to correlate with greater progression of retinopathy.<sup>38</sup> The progression slows or regresses after pregnancy; 6½-year follow-up studies indicated that

retinopathy in previously pregnant patients was similar to that observed in never pregnant controls.<sup>6</sup>

In contrast, pregestational diabetic nephropathy may not be adversely affected by pregnancy.<sup>6,36</sup> Although the albumin excretion rate in T1DM pregnant women increased in the intensive treatment group of the DCCT, it was not different from that in non-pregnant controls at 6.5 years of follow-up. Although microalbuminuria may worsen in pregnancy, it generally returns to baseline within a few months postpartum.<sup>45</sup> Maintaining glycemic levels and blood pressure close to normal are the best strategies to prevent progression of renal disease.<sup>36</sup> Patients with moderate to severe nephropathy early in pregnancy may progress and continue to do poorly postpartum. Purdy et al<sup>7</sup> reported that postpartum renal function in diabetic women with creatinine >1.4 mg/dL at the onset of pregnancy, declined permanently in 45%, transiently worsened in 28%, and remained stable in 27% of the women.

## FETAL OUTCOMES

Congenital malformations are 4 to 10 times more likely in offspring of diabetic women than in non-diabetic women. These anomalies account for the majority of the increased perinatal mortality associated with pregnancies complicated with diabetes.<sup>4,5,30</sup> The major congenital malformations include cardiovascular, neural tube and skeletal abnormalities (Table 2).<sup>46,47</sup> Renal abnormalities and hypospadias also occur at increased rates.<sup>30</sup> Spontaneous abortions are more frequent,<sup>5</sup> but the degree of increase is somewhat controversial. Hanson et al<sup>46</sup> reported a highly significant increase when the mother's glycemic control was poor (A1C >10.1%). Danish women with diabetes self-reported rates of spontaneous abortions at 17.5% compared to 10% to 12% in nondiabetic controls.<sup>48</sup> In the review of Strotmeyer et al,<sup>14</sup> 10% of the pregnancies in T1DM ended in stillbirth, compared to 0.6% of the sisters and 0.9% of the controls ( $p<0.001$ , Table 1). Similarly, Casson et al<sup>30</sup> reported a 5-fold increase in stillbirths in such pregnancies. In an audit of stillbirths in T1DM, Lauenborg et al<sup>49</sup> identified causes for stillbirths as DKA, chorioamnionitis, placental abruption, placental infarctions, severe IUGR, and thrombosis of the umbilical cord. The women with stillbirths had sub optimal glycemic control (A1C >7.5%) early in pregnancy more often than the women without stillbirths, 64% vs 33% ( $p<0.004$ ), and continued to have poor control during pregnancy. Maternal DKA was associated with a very high fetal mortality rate.<sup>37,38</sup> Other adverse outcomes of pregnancies complicated by diabetes include a higher rate of macrosomia, IUGR, neonatal respiratory distress syndrome, and shoulder dystocia.<sup>30,39</sup> Folate deficiency and inadequate weight gain are well established causes of neural tube defects, especially in poorly nourished adolescents.<sup>31-34</sup>

**Table 2. Congenital malformations in infants of diabetic mothers**

System	Malformation	Age of gestation*
Neurologic	anencephaly, holoprosencephaly, microcephaly	4
Skeletal	sacral agenesis, caudal agenesis	3
Cardiovascular	ventricular septal defect, transposition of the great vessels, patent ductus arteriosus, pulmonary stenosis	5-6
Renal	duplication of ureter, renal agenesis,	5
Other	hypospadias	4

\* weeks

Medications taken prior to conception, especially during fertilization and organogenesis, may have detrimental effects on the fetus. For example, angiotensin-converting enzyme (ACE) inhibitors may cause fetal oliguria, severe fetal hypotension, and osseous cranial anomalies.<sup>36</sup> Likewise, the use of the acne medication isotretinoin during pregnancy has been associated with very severe fetal abnormalities of the central nervous system, cardiovascular system, craniofacial formation, as well as parathyroid hormone deficiency.<sup>50</sup> Moreover, cigarette smoking, drugs, and alcohol use may cause untoward effects on the fetus. Severe hypoglycemia may lead to maternal seizures and loss of consciousness which may cause automobile accidents and may result in neuropsychological problems, with electrophysiological impairment in the child.<sup>2,39,40</sup> Rebound hyperglycemia after hypoglycemic events is thought to be a cause of fetal macrosomia.<sup>40</sup>

## PRECONCEPTION CARE AND MANAGEMENT DURING PREGNANCY

A diabetic woman who wishes to become pregnant needs preconception advice and counseling. Before pregnancy, glycemic control should be maximized and the underlying disease should be assessed thoroughly. Preconception counseling in T1DM decreases the risk of congenital malformations, spontaneous abortions, and stillbirths (Table 3).<sup>11,12,47,51,52</sup> Although the data reported were in adult pregnant women, the information is applicable to adolescents. Malformation rates and mortality rates dropped from 14% to 2.2% and 7% to 2%, respectively during a 15-year period.<sup>51</sup> The rates began to rise when the program was discontinued. The T1DM women who received preconception counseling for a mean duration of 17 weeks prior to becoming pregnant had a reduced rate of congenital malformations compared with controls (1.2% vs 10.9%), though the level of glycemia in both groups was similar.<sup>11</sup> Thus, preconception intervention is most beneficial in positively impacting the critical periods of embryogenesis and organogenesis. Preconception care in diabetic adolescents, coupled with ongoing prenatal intervention, reduces the high rate of spontaneous abortions and improves infant outcome.<sup>12,52</sup> Glycohemoglobin levels at first diagnosis of pregnancy are lower in women who attend such programs and correlate with better glycemic control during conception and embryogenesis.<sup>11,12,52</sup>

The majority of teenage pregnancies are not intended and out-of-wedlock adolescent pregnancies are not well received in the United States.<sup>27,29</sup> Thus, preconception counseling is usually not applicable. The necessary intense care of a pregnant adolescent with T1DM is cumbersome and difficult, though when applied through the entire pregnancy it leads to better outcomes. A team approach to care for T1DM is most effective and should be instituted as soon as possible.<sup>11,12,47,51,52</sup> Prenatal care should include nutritional counseling and weight gain guidance based on the preconception body weight and adequacy of the nutritional intake to avoid hypoglycemia and ketosis.<sup>53</sup> Dietary habits and preferences need to be considered to facilitate compliance and to meet nutrient requirements. Although controversial, most agree in striving for euglycemia, while ensuring appropriate weight gain of the woman and fetus. Jovanovic allows for an intake of 40% calories as carbohydrate, 20% as protein, and 40% as fat, with the caveat that breakfast is small (less than 10% of total calories). These percentages should be adjusted for glycemic control, insulin usage, and level of activity. Others favor a more liberal intake of carbohydrates (45%-55%) as long as the premeal insulin dose is adjusted appropriately.<sup>53</sup> Much of the available data on dietary intake are from gestational diabetes studies without the specific concerns of T1DM adolescent patients who more easily experience hypoglycemia or ketosis and are not very compliant.

Prenatal vitamins including folate and calcium should be initiated as soon as pregnancy is diagnosed. Folate doses are often increased up to 5 milligrams in order to prevent the neural tube abnormalities frequently found in infants of T1DM mothers.<sup>54</sup> If there is any indication of drug, alcohol, or cigarette use, these should be discouraged and discontinued. Any prepregnancy medications such as diuretics, ACE inhibitors, or acne treatments should be stopped immediately. Antihypertensive medications that are safe in pregnancy should be started and adjusted to maintain tight blood pressure control (ie, calcium channel blockers).<sup>55</sup> Alpha-methyldopa and hydralazine are two antihypertensive medications that have been used more extensively in pregnant women.

Early photography of the fundi will serve as a baseline to assess the degree of retinopathy and may infer the degree of microangiopathy present. Close follow-up with an ophthalmologist is necessary at least every trimester, if baseline photographs are normal, and more frequently

if baseline photographs show any degree of abnormality. A detailed antenatal evaluation for diabetic women with nephropathy, including evaluation of serum creatinine, uric acid, urea nitrogen, creatinine clearance, and urine culture is important. Creatinine clearance and protein excretion should be assessed at least every trimester, and more frequently if abnormal. Monitoring for anemia (due to renal loss of erythropoietin or iron deficits) and of thyroid function (due to the high rate of coexistent autoimmune thyroid disease in patients with T1DM<sup>56</sup>), are recommended and appropriate treatment instituted at once to avoid possible poor outcome for the infant.<sup>57</sup>

Many authors have reviewed the targets for optimum glycemic control; however, a consensus has not been reached. Jovanovic recommends 1-hour postprandial whole blood glucose <120 mg/dL (6.7 mmol/L), and fasting blood glucose <90 mg/dL (5.0 mmol/L). The American Diabetes Association recommends <140 mg/dL (7.8 mmol/L), and <100 mg/dL (5.6 mmol/L), respectively.<sup>58</sup> The A1C levels should be checked regularly to ensure compliance with the program and to guide insulin doses and dietary advice. The rapid-acting insulin analogs have been shown to be safe in gestational diabetes, and preliminary data indicate that they are safe in T1DM pregnancies. However, there are no data on long-acting insulin analogs use in pregnancy. Furthermore, obstetrical care and diabetes care should be jointly agreed upon to maximize patient participation and outcome of the pregnancy. Regular follow-up visits, ongoing dietary counseling, and emotional and psychosocial support are needed. Plans for the newborn, including child rearing, adoption, or alternative care (eg, grandparents) should be initiated as early as possible. A plan for the adolescent to complete her education is another major issue best approached early. Counseling for the mother-to-be, the father of the baby (if available), and the future grandparents is recommended.

### PREGNANCY PLANNING AND PREVENTION

Due to the high risk nature of pregnancy in adolescents with T1DM, pregnancy planning and/or prevention should play a major role in their care. The focus should be on prevention of pregnancy and improving the sexual education of the adolescent population.<sup>10,28</sup> The American Diabetes Association recommends that all women with diabetes of child-bearing potential use appropriate contraception and receive counseling about the risk of malformations associated with unplanned pregnancies and poor glycemic control.<sup>55</sup> Unfortunately, contraception for teenagers has been politicized, thus without parental involvement and/or consent it may be difficult to obtain in the United States.

Table 3. Outcome of Pregnancies Complicated by Diabetes

Authors	Congenital Malformations		Spontaneous Abortions		<i>p</i> value
	Preconception care	Routine care	Preconception care	Routine care	
Dicker, et al <sup>52</sup>			5/59 (8.5%)	10/35 (28.6%)	<0.001
Steel, et al <sup>47</sup>	2/143 (1.4%)	10/96 (10.4%)			<0.005
Kitzmiller, et al <sup>11</sup>	1/84 (1.2%)	12/110 (10.9%)			<0.01
Rosenn, et al <sup>46</sup>	0/28	1/71 (1.4%)	2/28 (7%)	17/71 (24%)	NS, <0.04

and the United Kingdom—the countries with the highest teen pregnancy rates.<sup>27</sup> Contraception in diabetic young women can be accomplished with cooperation between the patient, primary care physicians, gynecologists, and endocrinologists or diabetologists.<sup>23</sup> The factors associated with consistent birth control use in diabetic women and women with phenylketonuria were social support and positive attitudes toward birth control.<sup>10</sup> Low dose combination hormonal contraceptive pills are recommended; these can be safely used in adolescent T1DM patients in whom vascular disease is a low risk.<sup>25</sup> Barrier methods and spermicidal agents may be less acceptable to teenagers, resulting in poor compliance.

Emergency contraception, the so-called morning after pill, is another consideration. Use of such preparations has been limited due to prescription requirements, fear of hormones, possible adverse effects, and misinformation on availability and use.<sup>59</sup> The proposal to switch levonorgestrel emergency contraception (approved for prescription use in 1999, sold under the brand name Plan B®, Barr Pharmaceuticals, Pomona, NY) to over-the-counter status was not approved in May 2004 by the US Food and Drug Administration. Plan B consists of 2 (0.75 mg) pills of levonorgestrel to be taken as soon as possible within 72 hours after unprotected sexual intercourse. The rate of pregnancy is 0.4% if treatment is initiated within 24 hours and 2.7% if given within 72 hours. There are extensive data on the safety of this medication, though specific data on adolescents with diabetes are not available. The most frequent side effects are nausea and menstrual disruption. During a 29 month period, between 2001 and 2003, 40% of 7774 callers to a telephone prescription service in North Carolina (designed to increase access to emergency contraceptive pills) were teenagers.<sup>57</sup> Adolescents with diabetes frequently depend on pediatric endocrinologists for their care, thus a prescription for Plan B emergency contraception should be considered in advance of the crisis which may follow unprotected sexual intercourse. Additionally, the option for termination of pregnancy should be presented in a factual manner to the young woman, regardless of her religious background, so it may be performed as early as possible.

There are other considerations that need to be addressed in the course of the treatment of the adolescent with T1DM, particularly the encouragement of daily use of folic acid supplementation, even in those who are not sexually active or when pregnancy is not a consideration.

## CONCLUSION AND SPECULATION

The adolescent diabetic woman struggles with daily reminders of her disease—multiple fingerstick glucose checks, insulin injections, and an uncertain future of possible complications. Although preconception care is preferable, most adolescents do not intend to become pregnant. Unplanned pregnancy can be avoided with education, support, and contraceptives, offered to the

adolescent by diabetic educators, parents, and physicians. If pregnancy does occur, timely institution of excellent diabetes and obstetrical care promises at least a brighter future for the young woman and the infant.

Future challenges for the physician caring for pregnant young women with T1DM may include use of the rapid-acting insulins, long-acting insulins, and insulin pumps. Furthermore, if islet cell transplantation continues to show promise, consideration for unplanned pregnancies in young women on long-term immunosuppressant medications will need to be addressed. Interesting new data on gestational diabetes using fetal growth ultrasound to manage a patient, rather than strict dietary control and stringent glycemic guidelines<sup>60</sup> may offer a useful approach in pregnant T1DM adolescents. Outcomes including caesarean rate, small and large neonates, hypoglycemia, and neonatal intensive care admissions, were equivalent. Perhaps the lighter the burden we place on the teenager to conform to medical guidelines, the better chance we have of dealing with rebellion. However, adolescents will always be adolescents, for generations to come.

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## ABSTRACTS FROM THE LITERATURE

### Celiac Autoimmunity, Celiac Disease and Growth

The objective of this study was to evaluate growth and clinical features of children who tested positive for antibodies associated with celiac disease (CD). A cohort of HLA-DRB1\*03-characterized newborns from 1234 families in Denver, Colorado were prospectively followed since birth for the development of IgA autoimmunity transglutaminase antibodies (TG). Clinical evaluation, growth, anthropometry and biochemical assessments, as well as small bowel biopsies were performed. There were 33 children who tested positive to TG; 18 of them completed the studies, underwent repeated testing and were compared with 100 pair-matched controls. The TG-positive children had antibodies detected at a mean age of 4.4 ( $\pm$  1.2) years and the mean age at clinical evaluation was 5.3 ( $\pm$  1.5) years. They had significantly lower z-scores for height, weight and BMI ( $-0.3 \pm 0.7$ ), but not for weight- or height-for-age. They also had decreased mid-arm circumference and mid-arm muscle mass area. TG-positive children experienced more symptoms which increased over time, including abdominal pain, constipation and irritability/lethargy and these were independently associated with decreased weight gain. Thirteen (72%) of the 18 TG children had small intestinal mucosa evidence

of CD (Marsh 2-3), 2 showed increased intraepithelial lymphocytes (Marsh 1), and 3 had normal biopsies. No relationship was found between copies of HLA-DRB1\*03 and biopsy scores. The authors concluded that screening for CD identified TG-positive children who demonstrated mild alterations in weight and body composition and reported more symptoms than control subjects. They also had intestinal mucosa evidence of CD.

Hoffenberg EJ, Emery LM, Barriga KJ, et al. Clinical features of children with screening-identified evidence of celiac disease. *Pediatrics* 2004;113:1254-1259.

**Editor's Comment:** This prospective study provided important data of the natural history of CD autoimmunity in a genetically susceptible population. It also discerned the clinical findings of TG-positive children and the small intestinal mucosa alterations. However, the response to a gluten-free diet was not reported; expert consensus panels require the assessment of the response to dietary therapy as important evidential data for the diagnosis of CD. Additionally, the nutrient intake or fecal-fat excretion was not reported. It is possible that children decreased food ingestion to minimize the discomfort of malabsorption, thus

contributing to decreased weight and body composition. The prevalence of CD in children ranges between 0.4% to 1.0%,<sup>1,2</sup> whereas the prevalence of CD autoimmunity was close to 3% in this genetically susceptible population. TG-positive children presented few if any symptoms, and the autoimmune markers had a lower predictive value (75%) of detecting small bowel evidence of CD.<sup>3</sup> Symptomatic CD patients are at risk of long-term consequences, including osteoporosis, lymphoma and other autoimmune processes,<sup>4,5</sup> though no data are available on the risks of patients with CD autoimmunity and silent disease. However, CD screening of at-risk patients is increasingly being done by pediatric endocrinologists in patients with T2DM, short stature, Turner or Down syndromes who have shown a prevalence of CD autoimmunity of up to 15%. Screening for CD is best accomplished by measurements of TG and endomysial antibody immunofluorescence IgA. Both have a high sensibility and sensitivity, whereas antigliadin antibodies do not. However, the case finding efforts need

to be tempered with the cost of labeling children with CD, with the realization that this disease is difficult to prove and that only half of the patients follow a strict gluten-free diet.<sup>6</sup> The benefits from early diagnosis and treatment of silent patients have not been demonstrated. Thus more research is warranted along with careful monitoring of height and weight progression of children with CD autoimmunity.

Fima Lifshitz, MD

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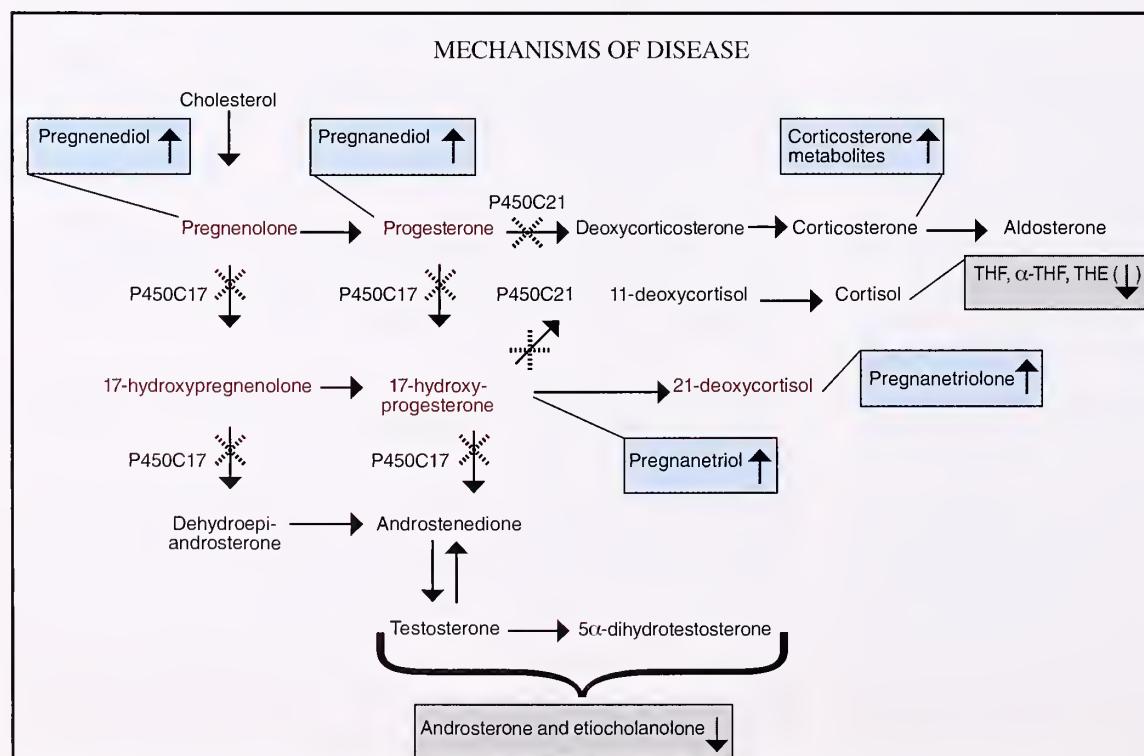
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## Congenital Adrenal Hyperplasia, Antley-Bixler Syndrome and Mutant P450 Oxidoreductase

Patients with biochemical evidence of apparent combined deficiencies of 17 $\alpha$ - and 21-hydroxylase have been recognized for almost 2 decades. The clinical features include infant females born with mildly to moderately virilized external genitalia in whom virilization does not progress post partum, to adult women with menstrual irregularities (amenorrhea). Affected males have normal external genitalia, cryptorchidism, or hypospadias. Serum and urine glucocorticoid and androgen values are

normal or low, while levels of precursors (progesterone, 17-hydroxyprogesterone, pregnenolone, pregnanetriol, pregnanetriol) are elevated. Recent analysis of the genes encoding the enzyme proteins (*CYP17A1*, OMIM 202110, chromosome 10q24.3; *CYP21B*, OMIM 201910, chromosome 6p21.3) did not disclose any mutations. The Antley-Bixler syndrome (ABS, OMIM 207410) is characterized by facial (midface hypoplasia with proptosis, choanal atresia, frontal bossing, dysplastic ears) and skeletal abnormalities (cranial, humero-radial and radio-ulnar synostoses, femoral and ulnar bowing, camptodactyly, joint contractures), and in some patients by genitourinary abnormalities (renal agenesis, vaginal atresia, virilization of female external genitalia, undemasculation of male genitalia). Heretofore, ABS has been primarily attributed to mutations in *FGFR2*.

Fluck et al and Arlt et al reasoned that perhaps the primary problem in patients with apparent combined deficiencies of 17 $\alpha$ - and 21-hydroxylase might be due to decreased levels of a common co-factor (Figure). Both enzymes require transfer of electrons to achieve the activated state. The electron donor is cytochrome P450



**Steroidogenesis and its impairment in patients affected by apparent combined P450C17 and P450C21 deficiency**  
Serum steroids reported in increased amounts are shown in red, and pathologically altered urinary steroid metabolites are shown in blue boxes if raised and gray boxes if reduced, linked to the steroid from which they are derived. Crosses indicate impairment of enzymatic activity.

(Reprinted with permission from: Arlt W, Walker EA, Draper N, et al. *Lancet*. 2004; 363:2128-35. Copyright ©2004 Elsevier.)

oxidoreductase (*POR*, OMIM 124015, chromosome 7q11.2), a flavoprotein that contributes electrons to all microsomal P450 enzymes. It does so by binding to NADPH through its flavin adenine dinucleotide (FAD) domain to which NADPH contributes 2 electrons; these electrons are then transferred to the flavin mononucleotide (FMN) domain of *POR* that, in turn, donates them to the target P450 enzyme. Mutations in *CYP17A1* that involve its binding to *POR* lead to decreased 17 $\alpha$ -hydroxylase activity. Accordingly, these investigators analyzed *POR* in 7 patients with combined deficiencies of 17 $\alpha$ - and 21-hydroxylase deficiency, some of whom had clinical and skeletal anomalies consistent with ABS. They found compound heterozygous or homozygous loss-of-function mutations in all patients including: 531T $\Rightarrow$ G: Tyr178Asp; 731+1G $\Rightarrow$ A: donor splice site intron 6; \*859G $\Rightarrow$ C: Ala287Pro; \*1370G $\Rightarrow$ A: Arg457His; 1475T $\Rightarrow$ A: Val492Glu; \*1706G $\Rightarrow$ A: Cys569Tyr; 1822G $\Rightarrow$ T: Val608Phe. The Ala287, Arg457, and Val492 mutations were in the FAD domain that binds NADPH and, predictably, changed steric conformation or charge leading to greatly reduced 17 $\alpha$ -hydroxylase and 17-20-lyase activities. The Cys569 and Val608 mutations were in the region that binds NADP<sup>+</sup> and resulted in less loss of enzyme activity. Patients with ABS tended to have the more severe mutations in *POR*, while those with the less severe defects only had disordered steroidogenesis. In no patient studied was a mutation in *FGFR2* identified.

Fluck CE, Tajima T, Pandey AV, et al. Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. *Nat Genet*. 2004;36:228-30.

Arlt W, Walker EA, Draper N, et al. Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. *Lancet*. 2004;363:2128-35.

**Editor's Comment:** Pregnant women who are heterozygous carriers of a loss-of-function mutation in *POR* may manifest gestational hyperandrogenism (acne, hirsutism) possibly due to the effects of both fetal hyperandrogenemia and impaired endogenous steroidogenesis. This resembles the hyperandrogenism seen in patients with a luteoma of pregnancy or placental aromatase deficiency. Arlt et al suggest that in the fetus with loss of *POR* activity, an alternate pathway of androgen synthesis is pursued: 17 $\alpha$ -hydroxyprogesterone is converted to 5 $\alpha$ -pregnane-3, 17 $\alpha$ -diol-20-one and the latter to androsterone by sequential actions of 5 $\alpha$ -reductase type I, 3 $\alpha$ -hydroxysteroid dehydrogenase, and low levels of 17 $\alpha$ -hydroxylase. Since this pathway disappears in early infancy, virilization does not progress. These data suggest that ABS is genetically heterogeneous; one type is due to loss of *FGFR2*, and is not associated with genital malformation; the second type is due to loss of *POR*. *POR* is required for activity of both adrenal and hepatic microsomal P450 enzymes. Indeed, in infants of mothers treated with the antifungal agent fluconazole, that inhibits ergosterol synthesis by interfering with lanosterol 14 $\alpha$ -demethylase activity, skeletal deformities resembling those in neonates with ABS have been observed. The skeletal deformities observed in children with deficiency of *POR* may reflect an error in this pathway that affects skeletal embryogenesis.

Allen W. Root, MD

## Prevention of Progression from Pubarche to Polycystic Ovarian Syndrome

There is evidence that girls with low birth weight (LBW) and precocious pubarche (prior to 8 years of age) are at high risk of polycystic ovarian syndrome (PCOS) even if not obese. Ibáñez and colleagues performed a randomized early prevention study in 24 such girls 6 to 12 months post-menarche. In each, precocious pubarche was diagnosed by high serum androstenedione and/or DHEAS levels. To be included in the study, girls had to have a birth weight for gestational age <1.5 SD, BMI <26%, hyperinsulinemia on a 2-hour OGTT (peak serum insulin >150  $\mu$ U/mL or mean serum insulin >84 mU/L), and subclinical ovarian hyperandrogenism (17-HO progesterone response >160ng/dL to GnRH agonist). They were randomized to receive either metformin 850 mg once daily or no treatment for 12 months. Serial clinical and biochemical measurements were made throughout the study.

There were no differences in any parameter between the treated and untreated groups at baseline. All subjects had increased androgen levels, abnormal lipid profiles, increased total body fat and reduced lean body mass. By 12 months, the treated group showed significant decreases in androgen levels, LDL cholesterol, and total body and truncal fat mass, and increases in HDL

cholesterol and lean body mass. In addition, insulin resistance was normalized. Most of these effects were seen between 3 and 6 months of treatment. The untreated group had significant worsening of each of these parameters. The authors conclude that the early post-menarchal years are an important period in the evolution of PCOS in girls with the predisposing clinical criteria. The authors also noted that the intervention was effective although limited to a once-daily medication without any other lifestyle change.

Ibáñez L, Ferrer A, Ong K, Amin R, Dunger D, de Zegher F. Insulin sensitization early after menarche prevents progression from precocious pubarche to polycystic ovary syndrome. *J Pediatr*. 2004;144:23-29.

**Editor's Comment:** This is an important and well-designed study performed by a group of investigators with significant research experience in this area. Their suggested pathophysiologic schema for the development of PCOS consists of girls with LBW but normal catch-up growth who maintain reduced muscle mass and become insulin resistant. This predisposes them to central obesity and excessive fat mass despite appearing lean, as well as to PCOS. Ibáñez and colleagues also suggest that their

data provided evidence that the endocrine-metabolic state is primary rather than secondary in this process. These are provocative conclusions and, if applicable to other patient populations, suggest an important role for insulin sensitizers, such as metformin, in the prevention of PCOS. Most pediatric endocrinologists are encountering more patients with PCOS. Therapy often includes metformin, an androgen-receptor blocker, and/or oral contraceptives,

but the results are rarely satisfactory. Clearly, there is a need to prevent the development of this syndrome. The etiology may not be the same in all cases, but close follow-up is merited in all girls born with LBW, as well as all girls presenting with premature pubarche. It is not unreasonable to suggest preventive therapy in some of these children.

William L. Clarke, MD

## Improving Accuracy of Linear Growth Measurements

A survey study of pediatric and family primary care practices in 8 areas of the United States found that 70% employed inaccurate techniques for measuring children.<sup>1</sup> As follow-up, Lipman et al analyzed the effectiveness of an intervention aimed at improving the accuracy of linear growth measurements. From the 259 prior practice responders, 8 per geographic area were randomly recruited and divided into intervention and control arms of the trial of 55 practices (44 pediatric and 11 family practice). Practices cared for an average of 4000 children, and employed an average of 3.6 staff responsible for the measurements (21% RNs, 23% LPNs, 56% nurses' aides/medical assistants) with an average of 8.2 years experience. At baseline, correct overall measurement technique was demonstrated on 30% of measurements. Proper equipment was used in 58% of standing measured children and in 18% of recumbently measured children. The measurements differed by an average of 1.2 cm within the same child by study staff (differences ranged up to 12.1 cm). The intervention group received: a written pre-test of knowledge of growth measurement, a slide presentation and handouts on both proper measuring techniques and the physiology/pathophysiology of growth disorders, the installation of accurate measuring equipment and demonstration (plus return demonstration) on the correct measurement of height

and length, and a written post-test assessment. The control group received no intervention. Measurement techniques were re-evaluated after 3 and 6 months in both groups. Accurate measurement in the control group remained at 37% at 3 months and 34% at 6 months. The intervention group increased the accuracy of the measurements to 55% at 3 months and 70% at 6 months. At conclusion, the intervention group's mean difference in measurement from study staff decreased to 0.5 cm.

Lipman TH, Hench KD, Benyi T, et al. A multicentre randomised controlled trial of an intervention to improve the accuracy of linear growth measurement. *Arch Dis Child*. 2004; 89:342-346.

**Editor's Comment:** Growth is the single most important indication of a child's health.<sup>2</sup> Growth monitoring is an integral part of pediatric care. The American Academy of Pediatrics has recommended that height and weight be measured at least at birth; age 2-4 days; 1, 2, 4, 6, 9, 12, 15, 18 and 24 months; and yearly through age 21.<sup>3</sup> It is disheartening that Lipman et al found high prevalence of incorrect techniques among pediatric and family practices. Even more disheartening is that 10% of pediatric practices and 40% of family practices did not measure children at every well-child visit.<sup>1</sup> This is a worldwide problem with a lack of equipment or trained personnel, inaccurate plotting

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and misunderstanding of the reference curves.<sup>4</sup> Likewise, a study of an academic pediatric clinic found that 35% of well-child encounters failed to plot growth measurements and/or document a growth abnormality.<sup>5</sup> This study demonstrated that an intervention program can effectively improve the accuracy of growth measurement in clinical practices, and that the improvement increased with time. Thus, rather than forgetting the lessons learned, continued use of proper technique and proper equipment reinforced and improved performance. The most common reason for practices refusing to participate in this interventional study was "provider unwillingness due to low importance assigned to linear measurements." The importance of proper technique and equipment cannot be overemphasized as lack of these may lead to missed or delayed recognition of growth failure or can lead to unnecessary investigation and

specialist referrals. As the authors pointed out, the current average inaccuracy exceeds the difference between the defined cut-offs for normal and abnormal growth velocities. (See Online Resources at [www.GGHjournal.com](http://www.GGHjournal.com) for links to growth charts.)

Adda Grimberg, MD

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## Stature and Psychosocial Adjustment in Adulthood

The Wessex Growth Study in the United Kingdom is a prospective, longitudinal school-based study that followed the physical and psychosocial development of short healthy students, and their average stature classmates from school until 18 to 20 years of age. This report represents the third in a series; the prior studies occurred when subjects were 7 to 9 and 11 to 13 years of age. The objective was to ascertain whether any psychosocial sequelae of short stature (during childhood or at the time of follow-up) could be detected in young adulthood. The short stature group (<2 SDs score for height) was compared to classmates of average height (10–90<sup>th</sup> percentiles). There were 48 short normal (SN) and 66 control (C) subjects; these were statistically indistinguishable on multiple sociodemographic variables. Ulph and colleagues used the Adolescent to Adult Personality Functioning Assessment (ADAPFA) to measure social and interpersonal role performance in 6 domains: education and employment; love relationships; friendships; coping; social contacts; and negotiations. Critical behaviors related to education received beyond school, employment status, relationships with a partner, parenthood, drug taking, drinking, and involvement with violence—referred to as activities of daily living—were also assessed. The data were analyzed with respect to both height at recruitment (ages 7–9) and as adults (ages 18–20). The participants were classified into 3 height groups: <2<sup>nd</sup> percentile (n=19); 2<sup>nd</sup>–50<sup>th</sup> percentile (n=61); and >50<sup>th</sup> percentile (n=34). The middle group consisted of both initial SN and C participants.

Height at recruitment was not associated with ADAPFA scores. The mean ADAPFA scores on 3 domains were (nonsignificantly) higher in the SN group, indicating poorer adaptation. (Gender and SES were significant predictors of several domain scores validating ADAPFA sensitivity.) There was no effect of adult height on outcome measures, nor was there a significant difference in the proportion of 3 adult height groups that received scores falling within

the clinical range. ADAPFA score was highest in the shortest group and for 2 specific domains. The measure of activities of daily living did not differentiate participants by recruitment or adult height. The authors conclude that healthy short stature adults did not have compromised psychological, social, or educational adaptation when sociodemographic variables were taken into account.

Ulph F, Betts P, Mulligan J, Stratford RJ. Personality functioning: the influence of stature. *Arch Dis Child*. 2004;89:17–21.

**Editor's Comment:** The Wessex Growth Study is the first and only prospective longitudinal study of social, educational and psychological adaptation of physically healthy short children from a community sample that employs a methodologically sophisticated approach. Because study participants were selected from schools, the referral bias that stems from recruitment through pediatric endocrinology clinics was obviated. Previous findings demonstrated that stature was not a statistically significant predictor of self-concept, behavioral or emotional functioning, or academic performance, although those with short stature were less satisfied with their height.<sup>1,2</sup> These earlier observations were reinforced by the current findings that adult stature was not a predictor of psychosocial adaptation. Importantly, statistical analyses in all waves of the study controlled for the influence of sociodemographic variables that are well-recognized predictors of quality-of-life outcomes, and which can be confounded with stature.

The authors conspicuously failed to mention that this cohort had also been examined during adolescence.<sup>3</sup> At that time, short boys reported being more than twice as likely as average stature boys to be the object of teasing, and much more likely to say that this upset them and that they spent break time alone. Short stature may thus place the individual at increased risk for psychosocial stress. However, the association between negative experiences

(teasing or juvenilization) and validated measures of behavioral and emotional functioning is relatively weak. Another study has shown the overall level of psychosocial adaptation of short youths derived from a clinic sample was comparable to that of the general population.<sup>4</sup> It can be inferred that, on average, short youths exposed to negative experiences adaptively cope so that signs of impairment do not emerge.

David E. Sandberg, PhD

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## The Marfan Syndrome -TGF- $\beta$ Connection

The Marfan syndrome (MFS) (OMIM 154700) is one of the original connective tissue disorders described by McKusick and colleagues. Its cardinal manifestations are aortic dilatation and dissection, dislocation of the lenses and overgrowth of long bones caused by over 600 mutations in the gene encoding fibrillin-1 (*FBN1*). Most evidence has suggested that the presence of abnormal fibrillin-1 interferes with the functions of tissues in which it resides. For example, the disturbance might weaken the ability of the aortic wall to resist hemodynamic forces, of the suspensory ligaments in the eye to hold the lens in place, or of the perichondrium to restrain the linear expansion of growing bones. However, controversy has persisted over the existence of a second MFS gene locus. A large French family with features of MFS was reported for which linkage was established not to *FBN1* on chromosome 15, but to markers located on chromosome 3p24.2-p25; the locus was designated *MFS2* (OMIM 154705).

Mizuguchi et al evaluated a child with MFS and a complex *de novo* chromosome rearrangement that included a breakpoint at the *MFS2* locus at 3p24.2-p25. The gene encoding the TGF- $\beta$  receptor 2 (*TGFB2*) was disrupted and a point mutation disturbed its splicing in affected members of the French family. They identified 3 additional missense *TGFB2* mutations from analysis

of 3 French families and 10 unrelated Japanese patients with MFS who had no mutation or linkage to *FBN1*, and demonstrated that several of the MFS mutations resulted in loss of receptor function.

TGF- $\beta$  receptors belong to the serine-threonine kinase family of cell surface receptors. When activated, they recruit and phosphorylate serine and threonine residues on cytoplasmic proteins that propagate TGF- $\beta$  signals to downstream pathways in cells. TGF- $\beta$ s regulate many cellular processes including proliferation, cell cycle arrest, apoptosis, differentiation and formation of extracellular matrix. Type II receptors are thought to mediate growth inhibitory signals, and *TGFB2* is considered to be a tumor suppressor gene.

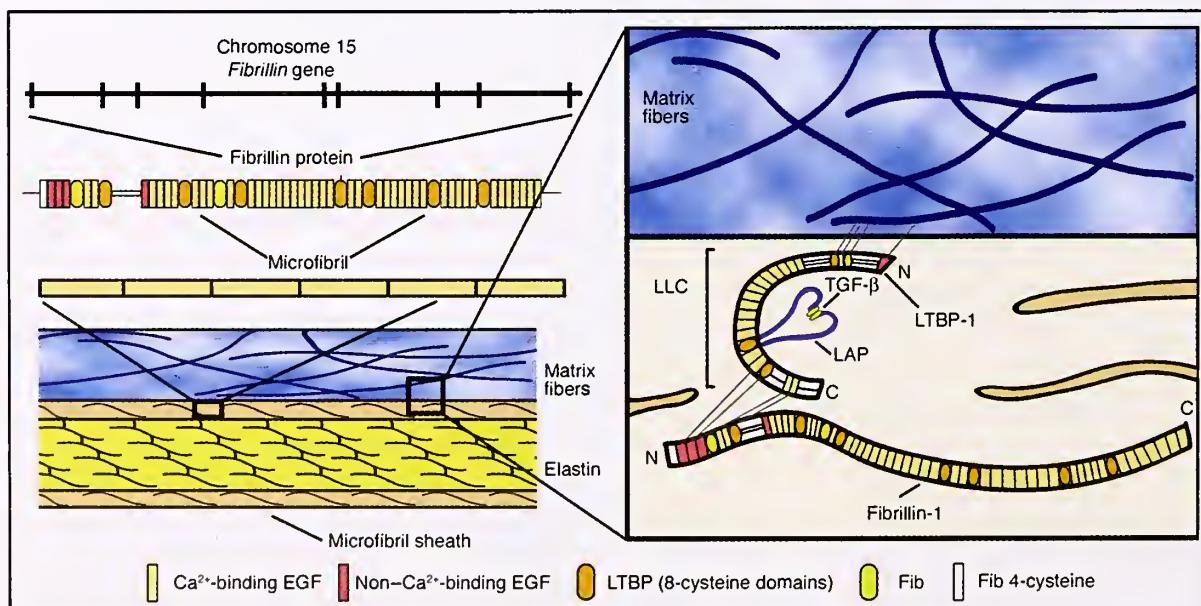
Byers described fibrillin-1 binding to so-called latent TGF- $\beta$  binding proteins (LBPs). These bind to inactive, latent TGF- $\beta$ s. In this way, fibrillin-1 can influence the extracellular availability of active TGF- $\beta$ . In MFS, the model suggests that mutations reduce the amount of fibrillin-1 in the matrix. The consequence is diminished sequestration of LBP-bound TGF- $\beta$  and a relative increase in the abundance of active TGF- $\beta$  in the matrix. In TGF- $\beta$ -responsive tissues, this results in exaggeration of processes that are regulated by TGF- $\beta$ , such as growth (Figure). Support for this idea comes from work recently published by Neptune et al, in which it is demonstrated that

emphysematous changes in the lungs of fibrillin-1 null mice could be partially ameliorated by antibodies that block TGF- $\beta$  function.

Mizuguchi T, Collod-Beroud G, Akiyama T, et al. Heterozygous *TGFB2* mutations in Marfan syndrome. *Nat Genet*. 2004;36:855-60.

Byers PH. Determination of the molecular basis of Marfan syndrome: a growth industry. *J Clin Invest*. 2004;114:161-63.

Neptune ER, Frischmeyer PA, Arking DE, et al. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. *Nat Genet*. 2003;33:407-11.



Fibrillin-1 is translated from mRNA encoded by *FBN1* gene on chromosome 15. (Reprinted with permission from: Byers PH. *J Clin Invest*. 2004; 114:161-63 Copyright ©2004 Am Soc Clinical Investigation.)

**Editor's Comment:** Taken together, these 3 papers strongly implicate TGF- $\beta$  playing a role in the pathogenesis of MFS. As suggested by these groups, the findings open up many new possibilities for treatment that focuses on excessive TGF- $\beta$  signaling as the target. These papers also highlight somewhat

of a renaissance in thinking about extracellular matrix and its components; they are not just static structural proteins, but dynamic elements that play important functional roles in development and disease.

William A. Horton, MD

## Somatostatin: New Effects on the GH-IGF Axis

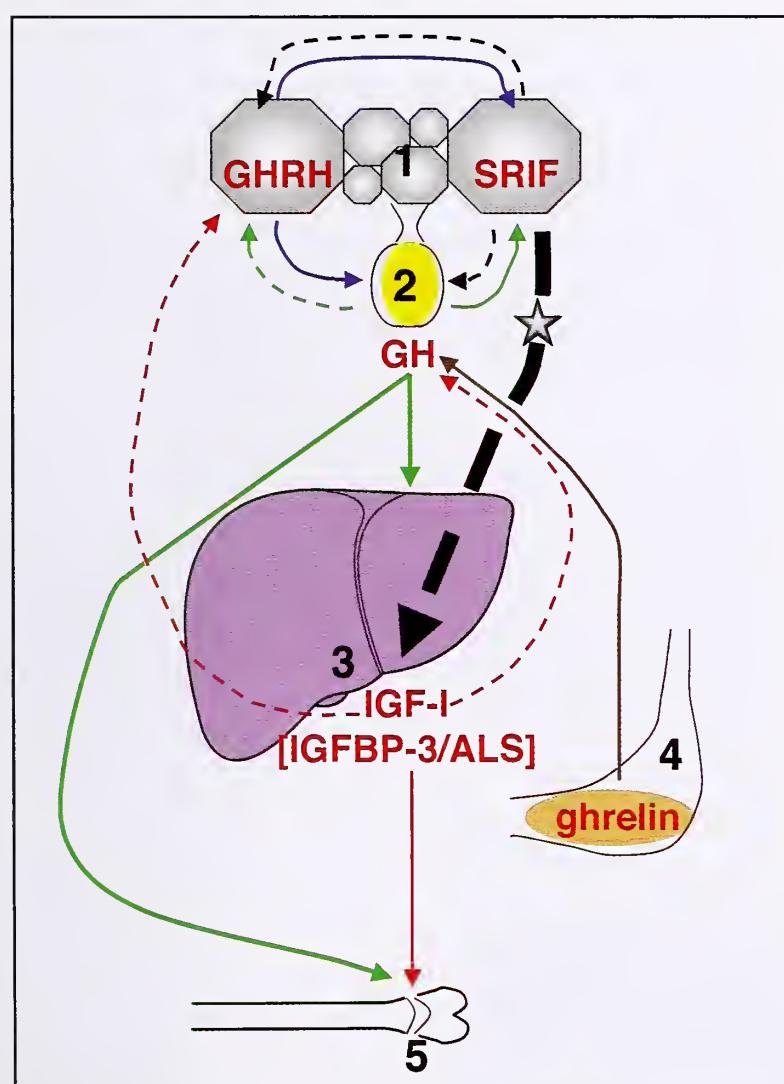
Somatostatin (SRIF) exerts multiple, mostly inhibitory, effects on endocrine and exocrine secretions, gastrointestinal function and cell proliferation. SRIF produced by the hypothalamic parvocellular neurons (paraventricular nucleus) inhibits pituitary growth hormone (GH) release and, subsequently, GH-induced insulin-like growth factor (IGF)-I production.<sup>1</sup> Murray and colleagues performed experiments that elegantly demonstrated peripheral inhibition of hepatic IGF-I production by SRIF and its analog, octreotide. RT-PCR of cDNA from isolated rat hepatocytes revealed expression

of both somatostatin receptor subtypes (SSTR) 2 and 3. IGF-I mRNA expression and protein secretion by isolated rat hepatocytes increased in a dose-dependent fashion after incubation with GH. This effect was inhibited by pretreatment with SRIF or octreotide, neither of which affected IGF-I levels without GH, nor affected other GH signaling pathways like phosphorylation of extracellular signal-related kinases (ERK) or induction of c-myc. SRIF or octreotide pretreatment decreased binding of radiolabeled GH to hepatocytes, and also decreased phosphorylation and nuclear localization of STAT5b, the main pathway by which GH induces IGF-I. SRIF inhibition of GH-induced IGF-I required inhibitory G proteins (G<sub>i</sub>'s; frequently transduce signals from SSTRs) and involved protein tyrosine phosphatase (PTP) activation but not increased suppressors of cytokine signaling (SOCS) 2 or 3. Furthermore, inhibition of GH-induced IGF-I production was confirmed in perfused whole rat livers *ex vivo*. Both models clearly excluded any central actions of SRIF on the GH/IGF axis.

Murray RD, Kim K, Ren S-G, Chelly M, Umehara Y, Melmed S. Central and peripheral actions of somatostatin on the growth hormone-IGF-I axis. *J Clin Invest.* 2004;114:349–356.

**Editor's Comment:** This paper described a new mechanism of action of SRIF and its analogs: the peripheral inhibition of GH-induced hepatic IGF-I production (Figure). SRIF analogs are the primary medical treatment for acromegaly, a state of GH excess usually caused by GH over-expression by a benign pituitary adenoma.<sup>2</sup> IGF-I elevation may become discordant with GH suppression in some treated patients, and biochemical control may not correlate with clinical improvement. These findings oppose the dogma of SRIF analog action at the hypothalamic-pituitary level. The new data support additional, peripheral action of SRIF and its analogs in suppressing GH-induced IGF-I production. This not only has important implications for the treatment of acromegaly, but also for the potential use of SRIF analogs in the treatment of cancer.<sup>3,4</sup>

Adda Grimberg, MD



**The GH-IGF axis.** Pituitary (2) GH secretion is controlled by hypothalamic (1) GHRH (from the arcuate nucleus) and SRIF (from the paraventricular nucleus), by negative feedback from IGF-I (3) and by ghrelin stimulation (4). GHRH actions are shown by blue arrows, SRIF by black, GH by green, IGF-I by red and ghrelin by brown. All solid arrows signify stimulatory actions; dashed arrows are inhibitory. Both GH and IGF-I act on target organs such as the growth plates of long bones (5) to stimulate cell survival, differentiation and proliferation. The paper by Murray et al adds a new mechanism of action for SRIF: hepatic inhibition of GH-induced IGF-I production, indicated by the starred, thick-dashed arrow.

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## ADHD Treatment & Growth

The multimodal treatment study of 540 attention deficit hyperactivity disorder (ADHD) patients reported the intent-to-treat analyses of 7 to 9 year old subjects who were treated for up to 24 months. Four naturalistic subgroups were formed in accordance with their patterns of medication intake over 2 periods: the first 14 months and during the 14-24 month period (Med/Med, Med/NoMed, NoMed/Med and NoMed/NoMed). Exploratory mediator analysis was performed to assess the effects of changes of medication intake, changes in scores of medication effectiveness (symptom ratings of 5 conceptually distinct domains of function) and growth (height and weight measures). The behavioral effectiveness of the medication use was greatest among children who ingested medications throughout the 24-month observation period. Those who stopped taking their medication and/or those who never received it showed increasing behavioral problems. However, there was significant growth deterioration among those who took medication for the longest periods. Between the Med/Med group and the NoMed/NoMed group the mean difference at the end of 24 months was -1.94 cm in height growth suppression, being similar in the 2 periods of observation. The weight gain changes were larger during the initial phase (-2.5 kg) than during the second period (-1.22 kg). Similar growth deterioration was observed in the 2 other groups while they received medication, with improvement during the NoMed periods. The authors concluded that consistent treatment with stimulant medication was associated with maintenance of behavioral effectiveness but continued growth suppression.

MTA Cooperative Group. National Institutes of Mental Health multimodal treatment study of ADHD follow-ups: changes in effectiveness and growth after the end of treatment. *Pediatrics* 2004;113:762-769.

**Editor's Comment:** This paper is difficult to read; however, it provides important data obtained with sophisticated methodologies and statistical analyses. The cooperative group clearly documented behavioral benefits of ADHD treatment, but there were consequences of the stimulant medication on growth as well as substantial difficulties in compliance. The high rate of patients who did not adhere to the drug regimen allowed the formation and assessment of 4 naturalistic groups. It has long been debated whether these medications alter growth progression; this study clearly demonstrated that they do. The growth-suppression effect persisted as long as the medications were ingested. This study also provided evidence that treatment interruption limits growth-suppression effects. The somewhat larger body weight deterioration that was observed might be due to the anorexic effects of these medications. Suboptimal nutrition appears to be an underlying cause of reduced growth, an aspect that should be thoroughly investigated. For a particular ADHD patient with growth concerns, when the stimulant cannot be interrupted, the physician should attempt to overcome the decreased dietary intake and correct nutrient deficits to foster appropriate growth. The pediatric endocrinologist is increasingly seeing more of these patients and should be aware of this important paper.

Fima Lifshitz, MD

## Cornelia de Lange Syndrome – Gene Mutations

The Cornelia de Lange syndrome (CdLS) (OMIM 122470) is characterized by a typical face (synophrys, upturned triangular nose, thin upper lip, long philtrum, downturned corner of the mouth), impaired growth, developmental delay, limb reduction defects, and anomalous development of the heart, eyes and genitourinary tract. It occurs *de novo* or may be transmitted as a dominantly inherited trait with variable expressivity. By studying families in which there were 2 or more affected members with a chromosome

translocation or deletion, both groups of investigators localized the disorder to chromosome 5p13.1 and identified mutations in a gene termed "Nipped-B-like" or *NIPBL*. There were heterozygous missense, nonsense, deletion and insertion mutations of *NIPBL*, all of which would have resulted in a truncated or untranslated protein product. The normal product of this 47 exon gene has 2804 amino acids (termed by the Tonkin group "delangin") that likely act upon chromosomes as an adherin, linking

the interactions of promoters and enhancers of homeobox genes. Further studies revealed that the human gene and mouse homolog of *NIPBL* were expressed during gestation in the anlagen of the limbs, cranium and branchial arches, placenta, kidneys, liver, heart, skeletal muscle and thymus. Homologs of *NIPBL* were identified in flies, mosquitoes, worms, plants and fungi.



Characteristic features of CdLS (Reprinted with permission from: Krantz ID, McCallum J, DeScipio C, et al. *Nat Genet*. 2004;36:631-635. Copyright © 2004. Nature.)

Krantz ID, McCallum J, DeScipio C, et al. Cornelia de Lange syndrome is caused by mutations in *NIPBL*, the human homolog of *Drosophila melanogaster Nipped-B*. *Nat Genet*. 2004;36:631–635.

Tonkin ET, Wang T-J, Lisgo S, et al. *NIPBL*, encoding a homolog of fungal Scc2-type sister chromatid cohesion proteins and fly Nipped-B is mutated in Cornelia de Lange syndrome. *Nat Genet*. 2004;36:636–641.

**First Editor's Comment:** Mutations in *NIPBL* were found in approximately 20% of patients with the clinical manifestations of the CdLS who were examined, implying that this disorder is likely to be genetically heterogeneous. Other sites that have been linked to the CdLS are located on chromosomes 2q37, 10p13, and 14q24, but an abnormality in one or more of the genes in these regions has not been detected to date. It is likely that as mutations in other genes that lead to the CdLS are identified, our understanding of the genetic regulation of somatic differentiation will be greatly enlarged.

Allen W. Root, MD

**Second Editor's Comment:** The multiplicity of clinical features of CdLS, combined with reports of chromosomal rearrangements in some patients, had long suggested that CdLS might be a contiguous gene syndrome. These papers demonstrate that the clinical manifestations reflect the diverse functions of a single gene product—delangin—during development. Most of the reported cases have severe clinical phenotypes and mutations that predict full loss of function, such as frameshift mutations leading to premature stop of translation. However, in a few cases mutations predicted to alter some, but not all functions, ie, 3 bp deletion that would remove a single amino acid appears to produce milder features, suggesting that the manifestations reflect loss or alteration of specific functions related to different regions of delangin. Correlation of clinical findings with specific mutations in more patients, combined with experimental modeling of specific CdLS mutations in mice, should help to sort out the relationship between genotype and phenotype.

William A. Horton, MD

## Metabolic Syndrome in Obese Children

The metabolic syndrome (MS) described as a link between insulin resistance, hypertension, dyslipidemia, and type 2 diabetes mellitus (T2DM), with an increased risk of atherosclerotic cardiovascular disease, has been reported to have a prevalence of 6.8% among overweight adolescents and 28.7% among obese adolescents (NHANES III). In order to determine the effect of the degree of obesity on the prevalence of the MS and its relationship to insulin resistance, Weiss and colleagues studied 439 obese (BMI >97% for age and sex), 31 overweight (BMI 85%–97% for age and sex), and 20 non-obese children and adolescents (4–20 years of age) with baseline measurements of BMI, blood pressure (BP), plasma lipids, C-reactive protein, interleukin-6, and adiponectin. Oral glucose tolerance tests were performed as well. Degree of obesity was defined by BMI z-scores (moderately obese = 2.0–2.5 and severely obese >2.5). The overall prevalence of MS was 38.7% in moderately obese subjects and 49.7% in severely obese subjects. Glucose, insulin, insulin resistance, triglycerides, C-reactive protein, interleukin-6, systolic BP, and prevalence of glucose intolerance (defined as 2hr glucose of 140–200 mg/dL) increased with increasing obesity. HDL cholesterol and adiponectin decreased with increasing adiposity. Three factors explained 58% of the variance observed: obesity and glucose metabolism, dyslipidemia and BP. Through multiple regression analysis of risk factors associated with the syndrome, a significant risk included age, sex, BMI z-score, race or ethnic group, and insulin resistance. Each half-unit increase in the BMI z-score was associated with a significant increase in the risk of the MS. White children had a higher risk than black children, and girls had a lower risk than boys.

A 2-year follow-up study was performed in 77 children; 34 with and 43 without MS. At follow-up 24 of 34 children continued to have MS. The 10 who improved had a lower BMI initially, gained less weight over the 2 years, and had decreased insulin resistance. The MS developed in 16 of 43 children who did not meet the criteria at baseline; they had gained more weight than the others. Eight subjects developed T2DM, and all had impaired glucose tolerance at baseline. The authors conclude that MS is much more common than previously reported and that each element of the syndrome is adversely affected by increasing weight. Of particular concern were the markers of inflammation, interleukin-6 and C-reactive protein, which escalated with increasing obesity and presumably put these children at high risk for cardiovascular disease.

Weiss R, Dziura J, Burgert TS, et al. Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med*. 2004;350:2362–2374.

**Editor's Comment:** This manuscript presents some frightening data. The prevalence of MS, once a diagnosis reserved almost exclusively for adults, is very common among obese adolescents. Weiss et al showed that not only adolescents, but children meet the criteria for this diagnosis, and that markers of cardiovascular inflammation are present at a very young age. The progression to develop MS over 2 years as weight continues to increase is remarkable.

Despite the importance of the documentation that this manuscript presents, the findings and conclusions are not surprising to most physicians who provide care to America's young. Indeed, the epidemic of childhood obesity is evident to any observer of children. It is heartening that some federal research funds are now being made available to study this problem and that Medicare is beginning to recognize

*obesity as a medical condition. Regardless of the data documenting the prevalence of obesity and the morbidities and co-morbidities associated with it, the behavioral and societal interventions required to stop its progression have not been adequately addressed. Reduction in the incidence and severity of obesity will require more than medical*

*research—it will require significant input of pediatricians and family physicians, schools, media, and commercial enterprises. Being apprised of the seriousness of the problem is just another wake-up call.*

William L. Clarke, MD

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